MAIZE GENETICS COOPERATION

NEWSLETTER

80

July 25, 2006

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and
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Columbia, Missouri

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NOTE: The 49th Maize Meeting will be held at St. Charles, IL, March 22-25, 2007.

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I. FOREWORD

The Maize Genetics Cooperation Newsletter exists for the benefit of the maize community as an informal vehicle for communication. Its inception and continuation has been to foster cooperation among those interested in investigating maize. This cooperation has distinguished our field from others and as a consequence has moved it forward at a pace greater than would have occurred otherwise. Your submissions are encouraged to disseminate knowledge about our field that might otherwise go unrecorded.

Because maize is both a commercial species and a genetic model system, the danger exists that the sharing of research materials might be diminished. It is imperative for us to work together to prevent this from occurring. Certainly, basic findings should be transferred to the industrial sector and basic advances in industry should be shared with the academic community for the benefit of both. Published materials must be shared for research purposes with the only restriction being against commercial use.

We remind the readers that contributions to the Newsletter do not constitute formal publications. Citations to them should be accompanied by permission from the authors if at all possible. Notes can be submitted at any time and are entered into MaizeGDB. We set an arbitrary cutoff of January 1, 2007 for the next print copy, volume 81. Electronic submission is encouraged and is done by sending your contributions as attachments, or as text of an email, to MaizeNewsletter@missouri.edu. Submissions must require minimal editing to be accepted.

We encourage the community to carry studies of general scientific interest to the formal literature. However, there is a great need to share technical tips, protocols, mutant descriptions, map information, ideas and other isolated information useful in the lab and field. This year, we call special attention to a number of special reports: the Maize Meeting Steering Committee (see pages 108-113); the Maize Genetics Executive Committee Community Survey Results (see pages 114-117); the Maize Genome Sequencing Project (see pages 72-74); and the table of Anchored BAC Contigs (see pages 75-97).

This year, the assembly, correction of copy and posting to the WWW was performed by Erin Broocke, an undergraduate student in the School of Journalism, University of Missouri-Columbia. As in the past, Shirley Kowalewski has been responsible for final redaction and layout of the copy. She has performed this task with speed, precision and a great sense of humor. The maize community owes her much gratitude for her continued service in this capacity.

Mary Schaeffer (Polacco)
James A. Birchler
Co-editors

BEIJING, CHINA
Institute of Genetics, Chinese Academy of Sciences

Effect of space on leaf cell plasmadesma in maize (*Zea mays* L.)

--Zeng, M; Zeng, Z; Ji, H

In our previous papers, we described a significant influence on progeny of dried maize seeds exposed to a special environment, space. Variation and mutant types of qualitative and quantitative traits were obtained and observed. Effects, both positive and negative, on young plants, ears and kernels were observed. The changes for cell ultrastructure of young leaves have been reported (MNL73:6-10; Chinese Space Sci. Technol. 18(6):63-67). Some changes were observed for plasmadesmata, chloroplasts and mitochondria, in particular thickened plasmadesmata (Figures 1-6).

Plate Ia Plate IIb

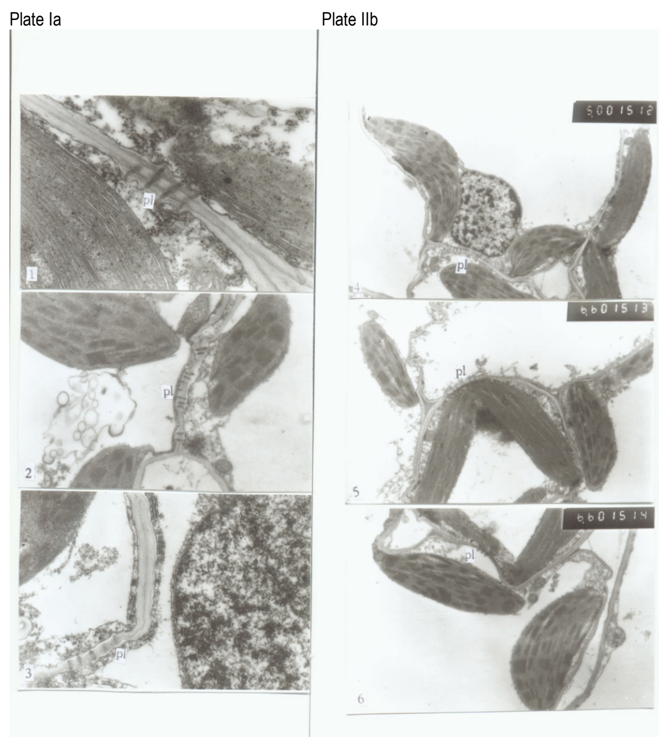

Plate Ia. Figures 1-3 show rich plasmadesmata between cell and cell, some chloroplasts or chloroplast and mitochondrion around the plasmadesmata, and swollen plasmadesma. (Fig. 1 X30000; Fig. 2 X39000; Fig. 3 X30000)

Plate IIb. Figures 4-6 show rich plasmadesmata between cell and cell, some chloroplasts or chloroplast and mitochondrion around the plasmadesmata. (Fig. 4 X4950; Fig. 5 X4950; Fig. 6 X4950)

According to our observations, we believe that space treatment of dried seed might cause an increase in the thickness of the plasmadesmata, and cause material to be exchanged between cells, further improving the level of metabolism.

BERGAMO, ITALY
Istituto Sperimentale per la Cerealicoltura

Characterization of *gl1*, a maize gene that affects cuticular wax accumulation

--Sturaro, M; Salamini, F; Schnelzer, E; Motto, M

The surfaces of land plants are covered with a cuticle secreted by epidermal cells, which plays several protective roles and consists of a reticulated cuticle membrane covered and interspersed by amorphous waxes (Kunst and Samuels, Progr. Lipid Res. 42:51-80, 2003).

The maize *glossy1* (*gl1*) gene is one of several loci involved in epicuticular wax biosynthesis in seedling leaves. Due to the strong reduction of juvenile waxes, mutations at this locus confer a glossy phenotype to the first five to six leaves, in contrast to the dull appearance of their wild-type counterparts. Although sequence analysis predicts a metabolic function for *gl1*, its specific activity in wax biosynthesis has not been defined yet.

To gain insights into *gl1* function, transcriptional analysis and microscopic inspection of mutant leaves were performed. From the expression profile it turned out that *gl1* activity is not restricted to the juvenile developmental phase of the maize plant, but it is active also in adult leaves and anthers (Figure 1). Moreover, *gl1* transcription is negatively affected by drought, although this stress condition promotes wax biosynthesis (Figure 2). These data suggest a broader role for *gl1* than anticipated on the basis of the

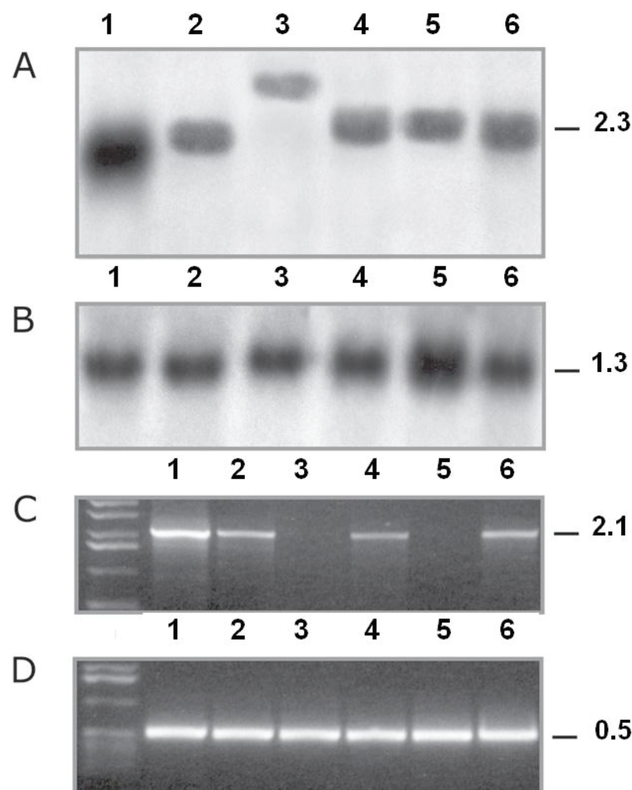

Figure 1. Analysis of *gl1* transcription. Northern analysis of *gl1* **(A)** and *cyGAPDH* **(B)** expression in: (1) young WT leaf, (2) young *gl1-Ref* leaf, (3) root, (4) old WT leaf, (5) silk and (6) anther. Expression in the same tissues of *gl1* **(C)** and *cyGAPDH* **(D)** as assayed by RT-PCR. Sizes in Kb are given on the right.

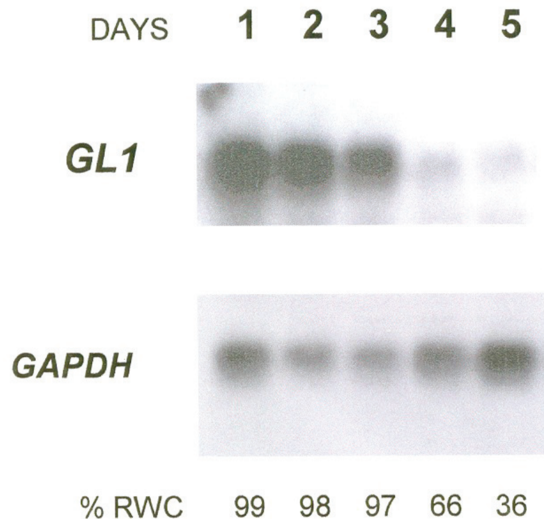

Figure 2. Effect of water stress on *gl1* transcription. Time course of *gl1* and cy*GAPDH* expression during a 5-day water stress period. RWC: Relative Water Content.

visual phenotype of mutant plants. Accordingly, ultrastructural analysis indicated a pleiotropic effect of the *gl1-Ref* mutation on juvenile epidermis development (Figure 3). In addition to the reduction in wax biosynthesis, scanning electron microscopy (SEM) analysis revealed alteration of leaf trichomes, namely decreased trichome size to half of the wild-type and increased trichome frequency. Transmission electron microscopy (TEM) analysis highlighted a strong reduction of cuticle membrane thickness in mutant seedlings. The effect of the *gl1* mutation seems to be limited to the epidermal layer; in fact, as revealed by light microscopic inspection, the whole architecture of mutant seedling leaves is not altered.

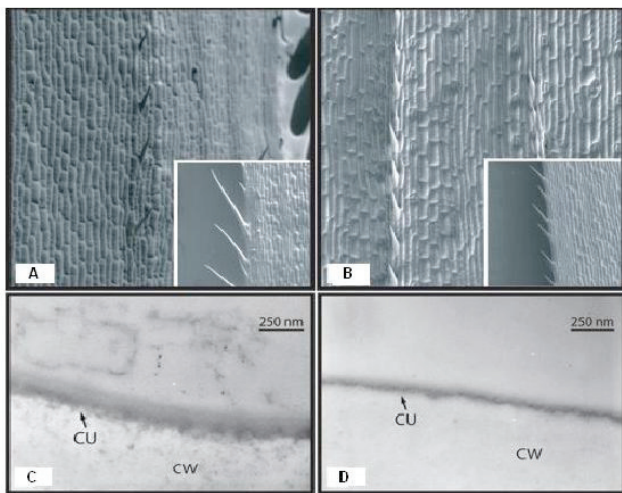

Figure 3. Ultrastructural analysis of seedling leaves. SEM analysis of WT (**A**) and mutant (**B**) leaf surface. Inserts: close-up view of upper surface showing details of trichome morphology and density (all images are at 100x enlargement). TEM analysis of WT (**C**) and mutant (**D**) cuticle membrane.

gl1 is the putative orthologue of the *Arabidopsis WAX2* gene given the strong homology of their sequences (62% identity at the protein level). The ultrastructural analysis of the *gl1-ref* mutant further supports this hypothesis; in fact, similarly to *gl1*, mutations of the *WAX2* gene alter cuticle membrane synthesis, epicuticular wax production and trichome morphology. Research is in progress in this laboratory to further clarify *gl1* functions.

The maize ribosome-inactivating protein b-32: role in the defence against fungal pathogens

--Balconi, C; Lupotto, E; Triulzi, T; Lanzanova, C; Gualdi, L; Conti, E; Motto, M

In maize endosperm, a cytosolic albumin with a molecular weight of 32 kDa, termed b-32, is synthesized in temporal and quantitative coordination with the deposition of storage proteins (Soave et al., Cell 27:403-10, 1981). Both cDNA and genomic clones encoding b-32 have been isolated. It was shown that the *b-32* genes form a small gene family (Hartings et al., Genet. Res. Camb. 65:11-19, 1995). The *b32* gene, as well as the 22 kDa storage protein zeins, are under the control of the seed-specific transcriptional activator *opaque2 (o2).* In *opaque2* mutants the b-32 protein is expressed at very low levels (Lohmer et al., EMBO J. 10:617-24, 1991). Although the role of b-32 in maize endosperm remains unclear, this protein has homology with several previously characterized ribosome-inactivating proteins (RIPs). It was found that b-32 is a functional RIP by the criteria of inhibition of in vitro translation in a cell-free rabbit reticulocyte system and specific N-glycosidase activity on 28S rRNA (Maddaloni et al., J. Genet. Breed. 45:377-80, 1991; Bass et al., Plant Cell 4:225-34, 1992). Additional evidence indicated that transgenic tobacco plants expressing b-32 showed an increased tolerance against infection by the soil-borne fungal pathogen *Rhizoctonia solani* (Maddaloni at al., Transg. Res. 6:393-402, 1997).

Research is in progress in our laboratories to verify if maize plants expressing b-32 in various organs and tissues have an increased defence against fungal pathogens in comparison with plants expressing b-32 only in the kernel. For these purposes transgenic plants were obtained through genetic transformation using the vector pSC1b32 containing the *b-32* coding sequence of clone *b32.66* under the constitutive promoter *35SCaMV* and the cassette *ubi1-bar* for Basta herbicide resistance as a selectable marker. These plants (T0) derived from callus regeneration after in vitro selection were tested for resistance to the herbicide Basta. T0 (Tt) plants were pollinated by B73 (tt) plants to obtain T1 plants. T1 seedlings were sprayed with Basta at the 3-leaf stage, and resistant plants were grown to maturity in a controlled environment. T1 plants were self-pollinated and were fully fertile and set seeds. In addition, biochemical analyses at the stage of flowering showed expression of the engineered protein in b-32 transgenic plants in tissues likely to be target sites for fungal invasion: silks, rachis, brace roots and husks, in addition to leaf tissues (Lanzanova et al., MNL 77:7-8, 2003).

A set of progenies (SM1, SM3, SM4, SM16, SM19, SM20) PCR b32+ and western+, and the progeny SM8 PCR b32+ and western negative (i.e., the 35S-b32 cassette is integrated but b-32 is not expressed) have been used in our studies. A detailed analysis of b-32 expression in leaves and pathogenicity tests were

performed on the progenies indicated above. Twenty seeds from each SM progeny were sown in a controlled environment and the tt plants eliminated by Basta spraying. Tt and/or TT plants were raised to maturity in a containment greenhouse. At flowering, two individuals for each progeny were analysed for the expression of b-32 in leaf tissues and for response to *Fusarium verticillioides* attack. The expression of b-32 was analysed in immuno-blot assays. As expected, in SM8 progeny b-32 expression was not detected; therefore, this progeny represented our negative control for pathogenicity tests. On the other hand, all the other progenies tested were b32-western positive. Comparison of b-32 expression among various individuals was performed after immuno-blot imagine scanner acquisition, using IMAGINE MASTER 1D Elite Version 3.01 (NonLinear Dynamyc Ltd) software. A differential b-32 expression in the various progenies was recorded; SM1 progeny showed the highest b-32 expression (SM1.1, the individual with the most abundant b-32 content in leaves, was chosen as reference for b-32 relative abundance in leaves); SM3.1 showed 89% b-32 content in comparison to SM1.1; SM20 and SM4 progenies had around 70% and 50% b-32 content, respectively, in comparison to SM1.1. All individuals belonging to SM16 and SM19 progenies possessed a b-32 content lower than 25% with respect to SM1.1.

This analysis allowed the identification of progenies with high (SM1, SM3, SM20), intermediate (SM4), and low b-32 (SM16, SM19) expression in leaves at the flowering stage; this is a useful range of expression for pathogenicity experiments to evaluate a differential response to *Fusarium* attack in leaf tissue colonization bioassays. For this purpose, at flowering stage, leaves of SM8 progeny not expressing b-32 protein were collected to determine the optimal spore concentration to generate a detectable *Fusarium* attack. *Fusarium verticillioides* (MRC826 strain, supplied by PRI-Wageningen) was grown on Potato Dextrose Agar (PDA) plates at 26°C until the mycelium covered the surface of the plate and used for fresh spore inoculum production. Leaves were surface sterilized and segments (1 cm squares) were dissected. Four leaf squares were plated on PDA and inoculated with a 5µl spore suspension at four different concentrations: (10^4, 10^5, 10^6 and 10^7/ml). At least three replicates for each experimental condition were considered. Infection progression was detected at different days after inoculation; mycelial growth was monitored daily, measuring fungal colony diameter around inoculated leaf squares. Control leaf squares were non-infected or treated with sterile water. This preliminary experiment supported the choice of a 10^5 spore/ml concentration, and of 3-5 days after inoculation as the detection time, for a reliable evaluation of the responses. Using this spore concentration, fungal growth on the leaves was gradually visible up to four days following the inoculation. Later on, the diameter was too large to be correctly measured because of colony confluence around the leaf squares plated together. In addition, when leaves were inoculated with suspension containing 10^5 spores/ml, mycelia were evident on the cut edges of leaves within 3 days, and later also on leaf surfaces. This last observation was not evident inoculating leaves with a spore concentration of 10^4/ml. Both controls, non-inoculated and sterile water-inoculated leaves, did not show any mycelial growth. Therefore, the protocol adopted for leaf tissue surface sterilization appeared to eliminate all external contaminations.

The bioassay parameters described above (5µl suspension containing 10^5spores/ml, 3-5 days following inoculation as detection time) were adopted for pathogenicity experiments including the negative control SM8, in addition to individuals of progenies previously tested for b-32 expression in leaves. Results indicated that the negative control SM8, when evaluated 4-5 days after inoculation, was more susceptible to Fusarium attack, in comparison to all the other progenies tested. At this stage, fungal colony diameter measured around the inoculated leaves of SM8 was significantly larger than that observed in progenies expressing b-32.

A good correlation between b-32 content in the leaves and level of resistance to *Fusarium* attack was observed. Individuals belonging to SM16 and SM19 progenies, with a b-32 content in leaves lower than that detected in the other progenies, appeared to possess a level of resistance to *Fusarium* significantly lower, showing high mycelial growth around inoculated leaves (colony diameter ≥15 mm, 5 days after inoculation). In addition, for these progenies (SM16 and SM19) and for the negative control SM8, a clear extension of mycelial growth on leaf surfaces was observed 3-4 days after inoculation. In the case of progenies with high (SM1, SM3, SM20) or intermediate (SM4) b-32 content in the leaves, in addition to a reduced mycelial growth on the cut edges of the leaves (colony diameter ≤10mm), a reduced growth on leaf surfaces was observed, even more than 5 days following inoculation. Experiments are in progress to extend pathogenicity tests to other plant tissues and to evaluate the specificity of b-32's role in the defence against other fungal pathogens (i.e., *Aspergillus*, *Penicillium*).

*This work has been developed within the framework of the EU-funded project SAFEMAIZE (ICA4-CT2000-30033) in FP5.

COLUMBIA, MISSOURI
University of Missouri

A trans-acting factor required for non-disjunction of the B chromosome is located distal to the TB-4Lb breakpoint on the B chromosome

--Lamb, JC; Han, F; Auger, DL; Birchler, JA

At the second mitotic division during pollen development, the B chromosome is not distributed to both of the daughter cells. Instead, at a high frequency one sperm cell receives both of the sister B chromatids and the other sperm gets none. The mechanism that causes this phenomenon, called non-disjunction, is not known.

In a reciprocal translocation stock involving A and B chromosomes, the chromosome with the B centromere (the B-A chromosome) is subject to non-disjunction, but not the reciprocal translocation element containing an A centromere and the distal portion of the B chromosome (the A-B chromosome) (Roman, Genetics 32:391-409, 1947). In order for the B-A chromosome to non-disjoin, an A-B chromosome must be present in the same cell, suggesting that a trans-acting factor resides near the distal tip of the B chromosome long arm (Roman, Genetics 35:132, 1950).

Translocation TB-4Lb resulted from an interchange involving the long arm of chromosome 4 and the B chromosome (Beckett, MNL 56:47, 1982). The breakpoint on the B chromosome is very near the tip of the long arm so that the B-A chromosome contains almost the entire B chromosome (Figure 1). To determine whether

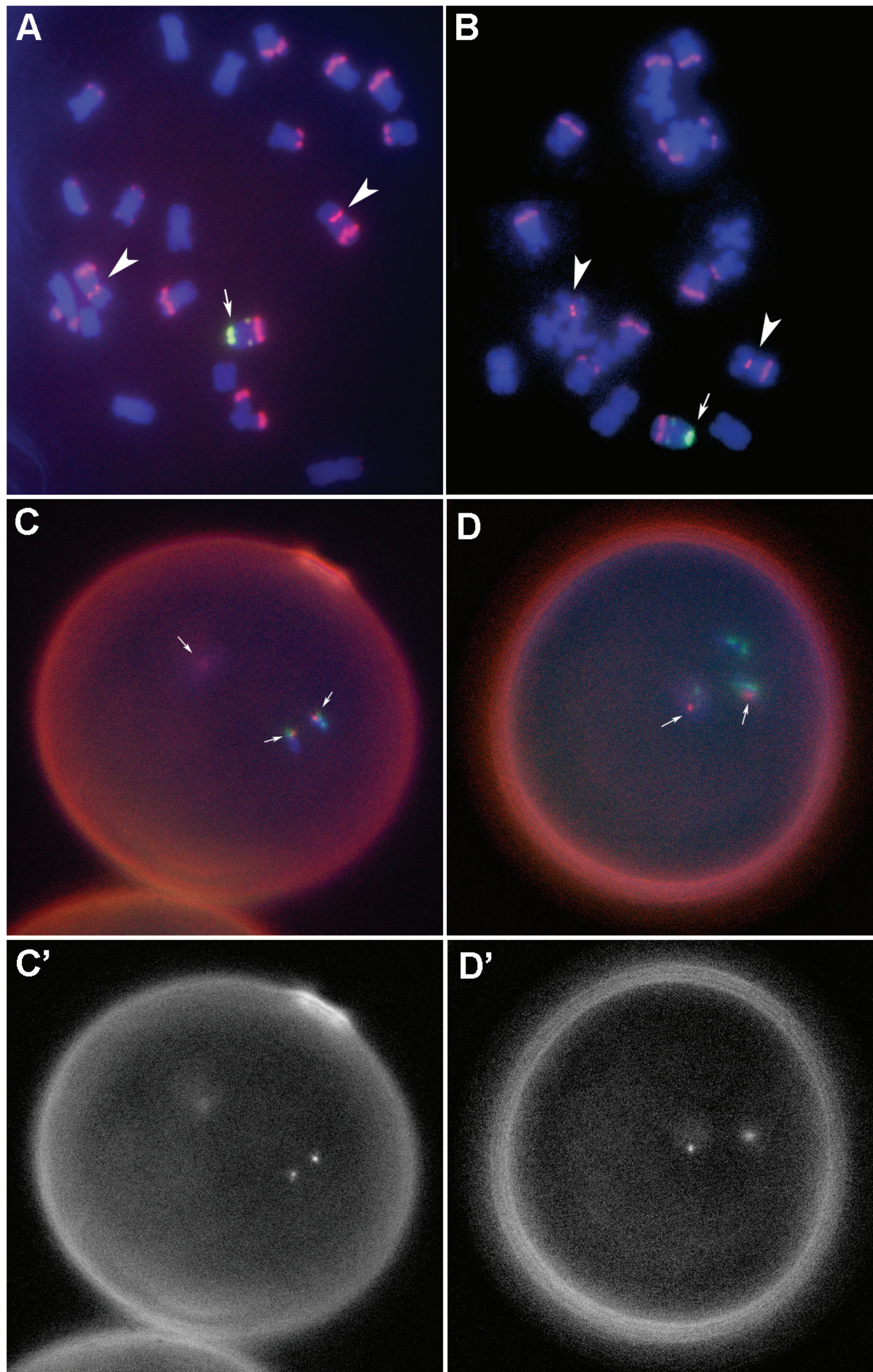

Figure 1. (A) shows a mitotic chromosome spread from a TB-4La tertiary trisomic plant that is hybridized with Cent4 in red and the B chromosome specific element, ZmBs, in green. The arrow heads indicate the location of the Cent4 element and the arrow points to the centromere of the TB-4Lb chromosome. Cent4 also hybridizes to the 180 bp knob element allowing confirmation that two intact copies of chromosome 4 are present (The knob on 4L is labeled with Cent4). (B) is a mitotic chromosome spread from a euploid heterozygote TB-4Lb plant labeled with the same probes as (A). The 4Lb-B translocation chromosome is identified by the smaller size and absence of the knob on the long arm. In (C), FISH was performed on the pollen from the individual in (A) using the 180 bp knob repeat (in green) and the ZmBs element (in red). All three nuclei are labeled by the ZmBs element (seen more clearly in (C') where only the red signal is presented) indicating that nondisjunction did not occur in the development of this pollen grain. (D) shows FISH on a pollen grain from the individual in (B). About half of the pollen from the euploid heterozygote individuals which had a ZmBs signal showed non-disjunction and about half did not consistent with expectations.

the physical location of the trans-acting factor on the B chromosome long arm was proximal or distal to the breakpoint, we tested the ability of the B-4Lb chromosome to undergo non-disjunction without the reciprocal 4Lb-B chromosome.

B-4Lb is particularly interesting because its breakpoint is distal to a small region near the tip of the long arm of B that is enriched for the B-specific sequence (Lamb et al., Chromosoma 113:337-349, 2005) (Figure 1), which is primarily present in and around the B centromere (Alfenito and Birchler, Genetics 135:589-597, 1993; Jin et al., Plant Cell, 2005). This B-specific region could be involved with the trans-acting effect on the action of the B centromere, given their similarity in sequence. If non-disjunction results from an interaction between the two sites of B specific repeats, then the B-4Lb chromosome should exhibit non-disjunction in the absence of the 4-B chromosome.

For many B-A translocations, including TB-4Lb, pollen containing the B-A chromosome and a normal chromosome 4 will not succeed in pollinating a tester line because the A chromosome segment on the B-A chromosome is present in two copies, reducing the competitive ability of the pollen (Auger and Birchler, J. Hered. 93:42-47, 2002). Because plants cannot be recovered that contain the B-4Lb chromosome from a test cross using a plant with two intact copies of chromosome 4 and a B-4Lb chromosome, we performed fluorescent in situ hybridization on pollen grains to assay directly the ability of TB-4Lb to undergo non-disjunction.

Plants that contained two intact copies of chromosome 4 and the B-4Lb chromosome (called tertiary trisomics), as well as plants containing one intact chromosome 4, one B-4Lb and one 4-B chromosome (euploid heterozygotes), were selected from progeny of a euploid heterozygote crossed as a female by a tester line. The karyotype of each plant was determined by examining mitotic chromosome spreads using a combination of FISH probes made from DNA elements specific to chromosome 4 (Cent4), centromeres (CentC), and the B chromosome (ZmBs) (Figure 1). See Kato et al. (Proc. Natl. Acad. Sci. USA 101:13554-13559, 2004) for a further description of the probes.

Mature pollen from both genotypes was fixed in 3:1 acetic acid:ethanol for 24 hours and then stored in 70% ethanol at -20°C. Pollen was rinsed in 2XSSC and then suspended in a probe mixture containing the B-specific element (ZmBs) and the 180 bp knob repeat in 2XSSC, 50% formamide and heated to 95C for 5 minutes. After heating, the pollen was incubated in the probe mixture at 37C for 24 hours. The pollen was suspended in VectaShield containing DAPI to stain the DNA, dropped onto slides, and covered with a cover slip. After waiting one hour for the DAPI to penetrate the pollen walls, the slides were examined with a fluorescent light microscope and images captured using a Magnafire CCD camera.

Over 100 well-labeled pollen grains from both the tertiary trisomic and the euploid heterozygote were examined for the presence of the B-specific probe signal in one or both sperm nuclei. The 180 bp knob probe serves as a positive control to confirm that the probe had penetrated into and hybridized to the three pollen nuclei. In the tertiary trisomic, about half of the pollen contained signals from the B-specific element and in every case both sperm nuclei contained the B-specific signal. About half of the pollen from the euploid heterozygote contained the B-specific probe and of these, half of them showed signal in both sperm. The remaining quarter of the total pollen had signal in only one of the two sperm, which is an indication of non-disjunction (Figure 1).

The pollen FISH procedure allows direct visualization of the results of B non-disjunction in the pollen grain. This allowed us to determine that the B-4Lb chromosome alone is incapable of non-disjunction. This result indicates that the trans-acting factor responsible for B non-disjunction is distal to the breakpoint of TB-4Lb and does not involve the distal ZmBs region of the B chromosome (Figure 1A). Thus, the mechanism for B non-disjunction does not involve interaction between the centromeric and distal B-specific sequences, and the trans-acting factor resides at a very distal position on the chromosome.

Some more data on endosperm color and embryo form in relation to haploidy

--Coe, EH; Neuffer, MG

A cross was made of a *C1/C1-I y1* derivative of stock 6 times a haploid-inducing stock carrying *C1* and *Y1*. In this cross, the colorless kernels have no anthocyanin expression in the endosperm or embryo, and subtle differences in endosperm color will be recognizable, not only because the female parent contributes no yellowness but because its kernels are smoothly rounded and somewhat flattened. In addition, the embryo is well-exposed and is of generous size in this stock, which allows some evaluation of variations in the size and form of the scutellum portion of the embryo. From six ears, 298 yellow, anthocyaninless kernels were screened for darker vs. lighter yellow vs. the remnant (i.e., neither darker nor lighter) without reference to the embryo. After color selection, all of the kernels were viewed for any altered embryo form, e.g. pointed or distorted scutella. The seeds were planted in a flat and the seedlings were classified for normal vs. the morphology characteristic of haploids (typically haploids are smaller, more stiff-leaved, somewhat streaked, and narrower-leaved). The results, from 283 seedlings that could be clearly classified, suggest that lighter endosperm color and altered embryos may be indicative of haploidy, but that neither criterion is definitive. This applies to the cross between these two specific germplasms, and may or may not be generalizable. Some additional observations are described below.

	Darker yellow	Darker, embryo form altered	Lighter yellow	Remnant, embryo form altered	Remnant
Normal	20	2	14	9	193
Putative haploids	2	1	10	4	28

The above results contrast with our report in the 2005 MNL that endosperms associated with haploid embryos are darker yellow. That report was based primarily on crosses onto yellow, hybrid materials. Our 2005 note refers to earlier evidence against 4n endosperms being associated with haploid embryos because 4n endosperms are substantially reduced in size. In a related study of crosses of *C1* x *C1-I*, Sarkar and Coe (Genetics 54:453, 1966) found that the rate of loss events for *C1-I* was the same in endosperms associated with haploid embryos as in ones associated with diploid embryos, i.e., that *C1-I* dosage was the same.

Some additional experiments suggest that darker yellow endosperm is an inconsistent phenomenon. The haploid-inducing stock was crossed in the summer of 2005 onto about 40 ears each of B73, B55, Oh43, Mo17, and W22 inbred lines. In each cross the endosperm colors were uniform, showing no discriminative differences in yellowness. Crosses onto yellow hybrids also were

inconsistent in 2005. Considering that this autumn led to fast drying and maturation, it is possible that seasonal influences on physiological conditions affect endosperm color development in kernels with haploid embryos. It is also the case that genes influencing yellowness differ among strains, including alleles at *Y1* and at an array of other loci, and dominant diluting alleles like *Wc1*.

Complementations, allelisms, and placements of mutants

--Coe, EH

The following notes present follow-up tests on mutants that were earlier located to chromosome arms and were subsequently tested with SSRs by Chris Carson. A few were unplaced previously. I appreciate advice from Phil Stinard, Janet Day Jackson, and Marty Sachs in pointing out potential allelisms that I might overlook, and in cross-checking against their records.

Tests of *blh*-N495B* on 1S: this mutant is allelic to *pg15*. Both show pale green leaves that develop bleached necrotic crossbands. The new allele is weak but viable; the other is lethal. With the suggestion of Phil Stinard, allelism tests were conducted after map locations with SSRs suggested potentially related phenotypes, including *pg15*. The common phenotype suggests diurnal induction of necrosis. This allele is designated *pg15-N495B*. It complements *nec2* and *zb4*, and its phenotype is distinct from *zb3*, which was located near *tub1* (at 2.5 on IBM2, 0.61 conventional cM) by Rugen et al. (MNL 75:17-18, 2001). Tests with *zb3* and *zn2* (unplaced) were attempted, but failed because the mutants were unseen in tester families.

Because both have been mapped in independent experiments, map data can be merged as follows:

(*blh*-N495B* -0- *sr1*) in 61 testcross individuals. Had 1 recombinant occurred, the distance would be 1.6±1.6 cM. -- Hoisington, DA. 1984. MNL 58:82-84.

(*blh*-N495B* - 14±5 - *umc1568*) in 21 F2 recessive individuals (42 strands). --Carson, C. 2001, MMP.

(*pg15* -11±5- *umc1160*) -- Carson, C. 2001, MMP.

Mapping of *sr1* has been reported with SSR markers:

umc1177 – 24±7 - *sr1* - 14±5 - *umc1166* in 42 strands -- Carson, C. 2001, MMP.

The SSRs are on IBM2 chromosome 1 in this order at these coordinates (to aid in estimating <u>intervals</u>, division by 4 is applied as an approximation to conventional cM):

umc1177 10.50 (~2.6) (~12 on the Genetic 2005 map)

umc1160 108.30 (~27.1) (~29 on the Genetic 2005 map)

umc1166 133.60 (~33.4) (~34 on the Genetic 2005 map)

umc1568 141.80 (~35.5) (~39 on the Genetic 2005 map)

The indicated order and estimated distances are *umc1177* -17- (*umc1160, sr1, pg15*) -5- *umc1166* -5- *umc1568*. If the position of *pg15* is as much as 11 units to the left of *umc1160*, it may be to the left of *sr1*, which is roughly 14 units to the left of *umc1166*. In any event, *pg15* is placed near 29, the location assigned to *sr1* on the Genetic 2005 map.

Tests of *w18* in bins 1.09-1.11: this mutant complements *l17,* which maps in bins 1.09-1.11. The phenotype of *w18* seedlings is pale yellow (aka white-luteus) with pale green streaks at the leaf base. In selfs from crosses of *w18-N495A* (Stock 128A) onto both A619 and B73, seedlings are also faintly green. Complementation also has been found between *w18* (which is within 4 cM of *umc1306* at 880.84 on IBM2 neighbors, i.e., between ~195 and 205 on the Genetic 2005 map) and *w24* (which is about 3 cM to the left of *umc1446* at 781.60 on IBM2, i.e., near 175 on the Genetic 2005 map). Data place *l17* within 17 cM of *bnlg1347* at 933.09 in IBM2 neighbors, i.e., between ~192 and 226 on the Genetic 2005 map.

The phenotype of seedlings of *l17* is described as luteus yellow and crossbanded. In selfs from crosses onto both A619 and A632, the luteus seedlings are faintly green. The luteus trait is associated with pale yellow (lemon) endosperm, an association that has not been recorded previously.

Tests of *bl*-N43* (*blotchedN43*) in 1L: this mutant is allelic to *zb7* and can be designated *zb7-N43*. Complementation is found with *v22*, and complementation has previously been found with *pg16*. The phenotype of *zb7-N43* is pale green in seedlings, sometimes cross-banded; irregular, pale green, bleached areas occur in mature plants. The phenotype of *zb7* (*zebra7*, *zb*-N101*) seedlings is strongly cross-banded on green, with bleached crossbands in mature plants.

Because both have been mapped in independent experiments, map data can be evaluated together:

umc1335 umc1446 -15- *bl*-N43* -20- *fdx3*-- Carson, C. 2002, MMP.

bz2 -23- *zb7* -9- *gs1* -26- *bm2* 1988 -- Sisco, PH. 1988. MNL 62:124.

Mapping of *gs1* with RFLP markers has been reported as follows:

gs1 -2- *umc72b* - *phi1* -7- *bnl8.29a* - *bm2* - *acp4* -- Sisco, PH. 1989. MNL 63:140.

The markers on IBM2 neighbors chromosome 1 are at these coordinates:

umc1446 781.60 (~195.4) (~177 on the Genetic 2005 map)

bz2 787.20 (~196.8) (~178 on the Genetic 2005 map)

umc72b 920.88 (~230.2) (~205 on the Genetic 2005 map)

fdx3 1098.40 (~274.6) (~244 on the Genetic 2005 map)

The MMP data are compatible with the Sisco data, which contributed placement of *zb7* between about 197 and 201 on the Genetic 2005 map. The indicated order and estimated distances are *umc1446* – *bz2* -23- *zb7* -9- *gs1* -2- *umc72b*.

Tests of *l*-85-3457-40:* this "Phenotype Only" luteus seedling mutant has been placed with the following SSRs by association in recessive bulks in F2:

umc1396	548.40	(~137.1)	bin 1.06
umc1446	781.60	(~195.4)	bin 1.08
umc1306	880.84	(~220.2)	bin 1.09

Estimated location for the gene is between ~145 and 185 cM on the Genetic 2005 map. A prospect for test is *l17,* in bin 1.09-1.10 at approximately 209 +/- 17 on the Genetic 2005 map.

Tests of *et*-N868A* in bin 2.00-2.02: this mutant complements

rgh2 in bin 2.00-2.02. It also complements *et2* in bin 2.02-2.04. Previous tests have found *et*-N868A* and *stf*-N868B* to be allelic. The gene is assigned the symbol *et3,* alleles *et3-N868A* and *et3-N868B*. It is located within about 10 cM from *umc1265* at 77.70 on IBM2 (i.e., between ~10 and 30 on the Genetic 2005 map).

Tests of *cb2* (was *cb*-N652B*) in bin 2.02-2.03: this mutant complements *al1* in bin 2.01. The location of *cb2* is within about 15 cM from *ole1* at 216.50 on IBM2 (i.e., between ~40 and 70 on the Genetic 2005 map).

Tests of of *v*-5537* on 2L: new tests show that *v*-5537* and *v4* are allelic, a correction to prior information in 1972 Cooperators' notes in MNL. Their phenotypes are very similar. The mutant complements *v24* and *wlv1*. This allele can be designated *v4-5537*.

Because both have been mapped in independent experiments, map data can be evaluated together:

(*umc1635 bnlg1887* -7- *v*-5537*) -17- *umc1042* – Carson, C. 2002, MMP.

gl2 -38- *mn1* -16- *v4* -- Van Horn, J. 1968. MNL 42:156-157.

The markers are on IBM2 chromosome 2 in this order at these coordinates:

mn1	328.79	(~82.2)
umc1635	344.80	(~86.2)
bnlg1887	346.50	(~86.6)
umc1042	466.65	(~116.7)

The indicated order and estimated distances are *mn1* - *umc1635* - *bnlg1887* -7- *v4* -17- *umc1042*, compatible with the location of *v4* between 100 and 104 on the Genetic 2005 map.

Tests of *w*-N77*, *w*-N332*, and *w*-N1907* on 2L: these three mutants are allelic to *w3* and can be designated accordingly. Mapping data include:

w-N1907* -0- *bnlg1045* and *bnlg2077*

bnlg1887 -13- *w*-N77* -24- *umc1042*

umc1004 bnlg2077 -11- *w*-N332* -18- *bnlg1520*

The markers are on IBM2 neighbors chromosome 2 in this order at these coordinates:

bnlg1887	346.5	(~86.6)
umc1004	381.8	(~95.5)
umc1042	466.65	(~116.7)
bnlg1045	471.21	(~117.8)
bnlg2077	474.8	(~118.7)
bnlg1520	596.55	(~149.1)

These data are inconsistent for order, most probably because of the high potential for errors due to chance in small samples. The data applied to obtain *w3* placement for the Genetic 2005 map at 135 +/- 5 have contradictory distance estimates and are no more clear with the above data.

Tests of *o*-N999A* in bin 2.07-2.09: this mutant complements *dek4*, *dek23*, and *dek16* on 2L. Seeds have an opaque phenotype and tend to be reduced in size. The gene was previously given the symbol *o16* but this was held pending these tests. The symbol *o16* can be assigned. It is located within 20 cM of umc1604, which is at 523.50 on IBM2 (i.e., between ~137 and 177 on the Genetic 2005 map). Tests with *o*-N1242A* and *ptd*-N901A* in the 2005 season were unsuccessful.

Tests of *o*-N1195A* in bin 2.08-2.09: this mutant complements *dek23* in 2L. Prior tests have shown complementation with *o16*. Tests with *dek4*, *dek16*, and *o*-N1242A* in 2005 were unsuccessful.

Tests of *d*-N282* in 3.06-3.07: this mutant is allelic to *na1* and can be designated *na1-N282*. Linkage data have been obtained as follows:

umc1266 -17- *d*-N282* -27- *bnlg197*

Marker locations are as follows:

umc1266	411.60	(~102.9)
bnlg197	511.50	(~127.9)

By apportioning the interval, *na1* can be estimated to be at about 101 +/- 3 cM on Genetic 2005, compared to its current placement at 108 +/- 3.

Tests of *wl*-N1906* in bin 3.08-3.09: this mutant complements *wlu1, y10, et1, v33,* and *w19* in this region (*w19* is the designation given to the albinism expressed in sectors when the *a-x1* deficiency is uncovered). The symbol *wlu8* is assigned. Linkage data have been obtained as follows:

(*wl*-N1906* -19- *bnlg1182*) 2001

umc1102 -26- *wl*-N1906* 2002

umc1140 -12- *wl*-N1906* -12- (*umc1594*, *umc1136*)

Marker locations are as follows:

umc1102	312.80	(~78.2)
umc1140	609.20	(~152.3)
bnlg1182	738.68	(~184.7)
umc1594	828.90	(~207.2)
umc1136	835.91	(~209.0)

The indicated order and estimated distances are *umc1140* -12- *wlu8* -12- *umc1594*, and *wlu8* at about 720 on IBM2, i.e., between ~140 and 150 cM on the Genetic 2005 map. This places the gene near but not within the *a-x1* deficiency, which encompasses *a1* at 139.9 and *sh2* at 141.9.

Tests of *spt*-N1620B* in bins 4.03-4.05: this mutant complements *spt2*. The two phenotypes are distinct, *spt2* showing rounded green spots on a pale field or rounded pale spots on a green field, while *spt*-N1620B* shows irregular or rectangular yellow spots on a green field. The symbol *spt3* is potentially applicable, but deferred pending additional tests. Linkage data have been obtained as follows:

(*spt*-N1620* -17- *umc1117*)

Marker *umc1117* is at 218.50 on IBM2, placing *spt3* between ~33 and 67 on the Genetic 2005 map.

Tests of *v*-N1835* in bin 5.05-5.07: this mutant complements *vp2* and *v12*. The symbol *v36* is assigned. This mutant has a briefly expressed, white-luteus virescent phenotype. Linkage data have been obtained as follows:

(*v*-N1835 umc1019*) association in recessive bulks in F2, where the estimated range is 15 units around the marker. Marker *umc1019*, aka *umc126a*, is at 469.60 on IBM2 and at 121.00 on the Genetic 2005 map, placing *v36* between ~105 and 135 on the Genetic 2005 map.

Tests of *wl*-N1393B* in bin 5.05-5.07: this mutant complements *v36*, *vp2,* and *v12*. It has a white-lutescent phenotype. The symbol *wlu9* is assigned. Linkage data have been obtained as follows:

(*wl*-N1393B umc1591*) association in recessive bulks in F2. Marker *umc1591* is at 314.10 on IBM2, placing *wlu9* between ~75 and 105 on the Genetic 2005 map.

Tests of *mn*-N1536* in bin 5.04-5.05: this mutant complements *dek9, dek26, dek27, dek29 dek33, prg1,* and *ren1*. It has minia-

ture kernels with loose pericarp. The symbol *mn5* is assigned. Linkage data are as follows:

(*mn*-N1536 umc1224*) association in recessive bulks in F2, linkage within 15 cM. Marker *umc1224* is at 315.22 on IBM2 neighbors, and *mn5* is uncovered by TB-5La at ~97, placing *mn5* between ~96 and 106 on the Genetic 2005 map.

Tests of *al*-84-5020-32* on 5: this "Phenotype Only" mutant complements *pb4* and *ppg1*. It was unplaced prior to tests showing association with *umc1591* in F2 recessive bulks, placing it in bin 5.03-5.04 between ~75 and 105 on the Genetic 2005 map. Mutant seedlings are pale green, grainy-streaked, and sometimes cross-banded; plants are variably unthrifty, with albescent-grainy leaves. Tests were unsuccessful in the 2005 season with *crp2* (aka *hcf143*), which is uncovered by TB-5Sc (i.e., is between ~0 and 82 on the Genetic 2005 map) and has a pale green seedling phenotype. Tests were also unsuccessful with *csy1*, which is at 210.19 on IBM2 neighbors, i.e., at ~57 cM on the Genetic 2005 map, and has a luteus yellow seedling phenotype. The phenotypes and/or locations of *crp2* and *csy1* are distinct from those of this albescent mutant. The symbol *al2* is potentially applicable, but is deferred pending additional tests.

Tests of *fl*-N1145A* on 5L: this floury is allelic to *dek33* and can be designated *dek33-N1145A*. Both display floury, wrinkled or shrunken kernels. Linkage data for both mutations are as follows:

(*fl*-N1145A bnlg609*) association in recessive bulks in F2.

a2 -7- *bm1* -13- *dek33* -21- *pr1* – Neuffer, 1992. MNL 66:39

Markers are placed as follows:

bnlg609	500.70 on IBM2	(~126 on the Genetic 2005 map)
a2	295.04 on IBM2 neighbors	82 +/- 1 on the Genetic 2005 map
bm1	92 on the Genetic 2005 map	
dek33	98 +/- 3 on the Genetic 2005 map	
pr1	109 on the Genetic 2005 map	

Based on linkage with *bnlg609*, *dek33* should fall between ~110 and 140 on the Genetic 2005 map. The order and distance data are inconsistent -- indeed, several other sources of data for the placement of *pr1* show conflicts inter se, so a definitive map involving *pr1* and *dek33* awaits future resolution. Incomplete complementation tests of *dek33-N1145A* with *dek9*, *dek26, dek27, prg1,* and *ren1* did not reveal allelism but did not exclude it in any instance.

Placement of *vp*-86-1407-15* on 7S and test with *vp9*: this "Phenotype Only" mutant has been placed with the following SSRs by association in recessive bulks in F2:

bnlg2132	53.30	(~13.3)
umc1015 (php20569a)	300.00	(~75.0)
umc1426	47.8	(~11.9)

Estimated location for the gene is between ~25 and 30 cM on the Genetic 2005 map. This is a pale endosperm, viviparous-embryo mutant. Two tests have shown complementation with *vp9*, which is at ~48 on the Genetic 2005 map, but additional evidence should be obtained before designating a new viviparous in such close proximity.

Tests of *vp*-8113* on 7S: this mutant gave positive allelism tests with *vp9* and could be designated *vp9-8113* if further tests confirm it. Map data that can be merged are as follows:

(*vp*-8113 o2 bnlg1247*) associated in recessive F2 bulks, the mutant being within 15 cM of each marker.

o2 -7- *vp9*

vp9 -11- *gl1*

Markers are placed on IBM2 neighbors as follows

o2	122.40	(~30.6)
bnlg1247	186.3	(~46.6)
gl1	191.31	(~47.8)

On the recent Genetic 2005 map *o2* is placed at 41.0, *vp9* is estimated to be at ~48 +/- 2, and *gl1* at ~60 +/- 4 cM. The new data do not improve on the estimated location for *vp9*.

Tests of *v*-N829A* on 9S: this mutant complements *v31* and *w*-9000*. Tests with *yg2* were ambiguous, with clear complementation in two direct tests but possible allelism in a third. The phenotype of *v*-N829A* is very similar to that of *yg2*, and mutant pools show linkage within 15% of *bnlg1724*. Additional tests vs. *yg2* are needed.

Tests of *w*-9000* on 9S: this mutant complements the *wd1* and *pyd1* deficiencies, *w11*, *l7*, *ar1*, *pg12*, *w2*, and *yg2*. Mutant pools show linkage within 15 cM of *bnlg1810*, which is very near *c1*. A remaining prospect for allelism is *l6*.

The phenotype of seedlings of *l7* is described as luteus yellow. In selfs from crosses onto both A632 and B73, mutant seedlings are faintly green.

Tests of *yg*-N2021* on 9S: this mutant complements *pg12*, *ar1*, *v1*, and *v31*. Mutant pools show linkage within 15 cM of *umc1417*, which is near *gl15*. Remaining prospects for allelism are *w11* and *v30*.

Tests of *ij*-N504A* on 10S: this mutant is allelic to *sr3* and can be designated *sr3-N504A*. Its recombination has been reported to be 2% with *umc1336*, which is on the long arm of 10. Both *ij*-N504A* and *sr3* are uncovered by TB-10Sc, and *sr3* is 3 cM from T9-10b, which is at 10S.40. The reported low recombination of *ij*-N504A* with *umc1336* contradicts its TB placement data and placement of its allele, *sr3*.

Tests of *pg*-N1224C* on 10L: this mutant is allelic to *v29* and can be designated *v29-N1224C*. TB-10L20 uncovers *v29*. Mutant pools show linkage within 15 cM of *umc1640*, and pools for *v29* show linkage within 15 cM of *bnlg1360*. Both SSRs are in bin 10.07, 14 cM apart on the IBM2 2004 neighbors map, or near 3.5 cM apart on the Genetic map. The combined data suggest a change in placement of *v29* to 123 +/- 10 on the Genetic 2005 map.

DAEGU, SOUTH KOREA
Kyungpook National University and
the International Corn Foundation

Progress of maize research in North and South Korea and other countries of Asia and Africa

--Kim, SK; Yoon, NM; Kim ,HJ; Kim, YB; Lee, GH

The International Agricultural Research Institute of Kyungpook National University (KNU) in Daegu and the International Corn Foundation (ICF) in Seoul, South Korea have conducted the following research activities on maize in South Korea, North Korea, Vietnam, Cambodia, Laos, Mongolia, East Timor, Nigeria, Camer-

oon, Ghana, Benin, Mali, and Burkina Faso.

In South Korea, the team has developed sticky waxy corn and super-sweet corn for local consumption. Korean people prefer waxy more than super-sweet corn, probably because of its origin in the Far East. In 2005, four waxy and two super-sweet (*sh2*) hybrids (single crosses) were registered by KNU and marketed in South Korea. In North Korea, a total of 35,000 crosses were tested throughout North Korea from 1998 to 2002. The collaborative team between South and North Korea selected 27 hybrids. On-farm testing at the Cooperative Farms was conducted for three years. Selected outstanding hybrids will be used for F1 seed production in 2006 for a large-scale commercial cultivation in 2007. In addition to the joint-breeding program, Suwon 19 hybrid, developed by the senior author in South Korea in 1976 as the first single cross hybrid corn in Asia, has been widely grown in North Korea commercially. The total production of food in North Korea has been increased from 1.5 million tons (1997) to 4.5 million tons (2005). Corn is the staple food for 70 percent of the population of North Korea.

In addition, the ICF/KNU program has helped to develop locally adapted corn cultivars (open-pollinated) in Ben Tre Province in Vietnam, Cambodia (for downy mildew resistance), Laos and Nepal (open-pollinated and hybrid development), Mongolia (OP yellow for livestock and Vitamin A supplemented as food), East Timor (top crosses with downy mildew resistance). The program has selected outstanding and stable OP cultivars for Ben Tre Province where the Vietnam War was the most severe. Rice, coconut and sugar cane are the main crops, and corn is being developed for feed production and green corn. A new crop of corn shall be rotated with the rice crop.

In Africa, the SAFGRAD/KNU program, with support from the Korea International Cooperation Agencies (KOICA) and the Africa Union, has conducted on-farm demonstration trials of *Striga* + streak virus tolerant maize cultivars in eight countries in West and Central Africa. The program has tested IITA and Cameroon national program developed STR (*Striga* tolerance and resistance) cultivars (mostly OPVs) with legume crops. The STR + legume package is considered one of the sustainable packages for a long-lasting solution in combating the worst parasite, the *Striga* species, in Africa. The team found that sorghum is the major host for seed production of *Striga* species in Africa. STR materials are tolerant to both *Striga hermonthica* and *S. asiatica*. Although IITA, in collaboration with national programs and CIMMYT, solved maize streak virus (MSV) problems by 1986, when the IITA maize team received the CGIAR King Baudouin Agricultural Award for producing 100 streak resistant (SR) maize cultivars (OPVs and hybrids), still farmers in some countries of the East and Southern Africa regions (including Namibia, Botswana, and Kenya, etc.) have suffered MSV epidemics that cause a significant reduction in maize yield. The team found that farmers in many countries in Africa sow the long rainy season cultivars of maize during the short rainy season (with only two months of rain). The maize breeding technology employed by the team is only tolerance that is based on the co-survival principle, without any chemical spray. We do not select single gene resistance because it's against the co-survival principle between pests and hosts in nature. Based on 40 years of corn breeding experience in the developing world and the USA, the senior author asserts that host-plant resistance with QTL genes can be the most sustainable technology and the one most respectful of nature. The recent outbreaks of bird flu and several other environmental hazards might be caused by ignoring the co-survival tolerance principle. We must respect nature.

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Identification of sources of resistance against *Erwinia* stalk rot *(Erwinia chrysanthemi pv. Zeae)* among medium maturing inbred lines of maize

--Kalia, V; Basandrai, AK; Thakur, SK; Jarial, RS

Bacterial stalk rot caused by *Erwinia chrysanthemi* pv. *Zeae* (Sabet,1954) victoria is a challenging problem and the most destructive disease of maize in the outer Shivaliks of the northwestern Himalayas, covering states like Himachal Pradesh, Uttaranchal, Jammu & Kashmir and adjoining plain areas of states such as Punjab, Haryana and Uttar Pradesh, etc. The disease causes losses in maize production in many southeast Asian countries also. The disease was first reported by Prasad in 1930, but its importance was first realized in 1969, when a severe outbreak occurred in the Bahl valley of the Mandi district in Himachal Pradesh, India. Keeping in view the importance and seriousness of the disease, the present investigations were undertaken to find a stable source of resistance against the malady. Resistant varieties are needed as the most acceptable solution to avoid this major cause of loss in yield. Out of forty inbreds evaluated, none was found to be immune: one inbred (DKI-9770) was highly resistant (<10% incidence); fourteen inbreds were resistant (10-20% incidence); and the remaining twenty-five were susceptible to *Erwinia* stalk rot (Table 1). Inbred lines DKI-9770, DKI-9712, DKI-9740, DKI-9555, DKI-9727, DKI-9560, 94140-Farakkaba and CM-113 were found to be consistent in their reaction in both years of evaluation, and thus can be a stable source of resistance against ESR. Disease spread in many lines was found to be at variance from the ESR percent incidence (Table 2). It was observed that the spread of rot occurred sometimes in the major part of the inoculated node but without affecting the rind or vice versa. Thind and Payak (1978) reported resistance in CM-104 (white), CM-104,

Table 1. Grouping of various inbred lines on the basis of mean incidence for two years of *Erwinia* stalk rot under artificial inoculation.

Immune	Highly Resistant (<10%)	Resistant (10-20%)	Susceptible (>20%)
None	**1** *DKI-9770 (8.1)	**14** *DKI-9712(19.6) *DKI-9740 (15.6) DKI-111(19.8) *DKI-9555 (15.5) DKI-159 (19.3) DKI-9727 (10.1) DKI-9746 (15.5) *DKI-9560 (16.9) DKI-9764 (20.0) Pob 33-5-Amar (17.2) Pob-27 (Htr) (19.0) *94144-Farakkba (10.9) *CM-113 (12.7) *DMR-sr.5 (19.5)	**25**

*inbreds showing resistance during both years
Figure in parentheses is the mean ESR incidence

Table 2. Mean performance of various traits in resistant inbreds.

Sr. No.	Entries	Days to husk browning	Plant height (cm)	Cob height (cm)	ESR spread (1-5)	Grain yield (q/ha)
1	94144-Farakkaba	87.7	209.6	102.5	3.5	18.9
2	CM-113	84.3	206.6	105.6	2.1	38.1
3	DKI-111	85.0	144.2	71.8	2.7	35.5
4	DKI-159	85.0	194.0	93.4	2.7	42.8
5	DKI-9555	86.3	201.0	97.4	2.0	30.0
6	DKI-9560	82.0	178.5	88.1	2.9	32.4
7	DKI-9712	91.3	182.6	87.0	2.2	34.6
8	DKI-9727	87.7	172.0	89.7	3.0	20.2
9	DKI-9740	87.7	167.1	70.0	2.8	21.4
10	DKI-9746	86.7	176.4	87.9	3.5	26.6
11	DKI-9764	85.7	161.9	72.7	3.4	26.6
12	DKI-9770	87.0	163.4	81.6	2.2	21.2
13	DMR-Sr. 5	86.0	185.2	89.5	1.9	21.3
14	Pob-27(Htr.)	90.7	213.8	106.1	1.7	19.5
15	Pob-33-S-Amar	88.3	207.3	98.1	2.5	30.8
	CD (5%)	2.0	18.2	20.0	1.0	11.1
	CV (%)	1.5	5.9	13.6	23.3	22.7

CM-105 and CM-600 against ESR among the material screened, andthey suggested that resistance can be further upgraded by sib pollination among resistant plants in these lines. Ebron, Tolentino and Lantin (1987) reported eight lines to be resistant out of 107 maize accessions screened. Sah and Arny (1990) reported all forty-five maize cultivars evaluated to be susceptible. Data on grain yield shows that out of eight stably resistant inbreds identified above, DKI-9712 (34.6 q/ha), DKI-9555 (30 q/ha), DKI-9560 (32.4 q/ha), CM-113 (38.1 q/ha) and DKI-9764 (26.6 q/ha) have high grain yield and are thus argonomically superior (Table 2). These lines can be utilized in the breeding programme for developing hybrids after studying their combining ability. These lines are observed to have medium cob placement, except CM-113 which has tall plants (206.6 cm) and high cob placement (105.6 cm)

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Nomenclature of sucrose synthase genes and the gene products

--Chourey, PS

Many single gene seed or endosperm mutants are symbolized based on their phenotype. The simple nomenclature becomes problematic however once the corresponding gene product is identified, and more so thereafter with the usual discovery of its paralogs. A case in point here is the *shrunken 1* (*sh1*) seed mutant, which was first described by Hutchison (J. Hered. 12:76-83, 1921) based on the shrunken seed phenotype. The *Sh1* gene product was first identified and designated as the Sh1-protein (Schwartz, Genetics 45:1419-1427, 1960), which was later characterized as a sucrose synthase (Sus) (Chourey and Nelson, Biochem. Genet. 14:1041-1055, 1976). Subsequent studies led to two additional non-allelic s*us* loci, which have been designated as s*h1*, s*us1* and s*us3* and the encoded products as SH1, SUS1 and SUS3 (Carlson et al., Plant Mol. Biol. 49:15-29, 2002). There was however no s*us2* gene in this nomenclature; so, *sus3* is now called *sus2*. Accordingly, it is proposed here that the corresponding protein products of s*h1*, s*us1* and s*us2* should be designated, SUS-SH1 (or SH1), SUS1 and SUS2, respectively. Marty Sachs has agreed to incorporate this nomenclature on the MaizeGDB website.

The mutations *sh2-i* and *sh2-N2340* share an identical intron splice site mutation and are most likely the same allele

--Clancy, M; Hannah, LC

The mutant alleles *sh2-i* and *sh2-N2340* were generated by Dr. M. G. Neuffer using EMS mutagenesis. The mutation *sh2-N2340* is available from the Maize Stock Center. These two mutants condition an intermediate or leaky phenotype. Mature kernels are less severely collapsed compared to the reference allele *sh2-R*. Sequencing established that *sh2-i* contains a G to A transition at the 3' terminus of intron 2 (Lal et al., Plant Physiol. 120:65-72, 1999). Approximately 10% of *sh2-i* transcripts are correctly spliced utilizing the mutant intron splice site. This generates a low level of adenosine diphosphate glucose pyrophosphorylase activity that results in the intermediate kernel phenotype.

Because *sh2-i* and *sh2-N2340* kernels are visually so similar and trace to the same source, we asked whether they contain the same mutation. Young shoot material was harvested from germinating *sh2-N2340* kernels, and genomic DNA was prepared using Plant DNAZOL Reagent (Invitrogen). DNA spanning exons 1 through 4 was amplified via PCR using the primers described by Lal et al. (1999). Sequencing of the PCR product established that *sh2-i* and *sh2-N2340* share the same G to A transition of the final nucleotide in intron 2. Hence, it appears most likely that the same mutation bears two different designations.

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Segregation of resistance to southern corn rust in set M RIL population

--Ji, HC; Brewbaker, JL

Southern corn rust (*Puccinia polysora* Underwood) becomes severe in the wet, somewhat cooler winter months (mean temp. 72ºF) of Hawaii. Previous evaluations of several hundred tropical and temperate inbreds revealed three distinct levels of general resistance (not racially-specific)—highly susceptible, highly resistant and intermediate. Highly susceptible lines (e.g., North American sweet corns) have lesions on all leaves and often fail to set seed. Some inbreds (normally tropical in origin) were highly resistant, showing very few lesions and only on the upper surface of lower leaves. An intermediate level of tolerance was also common among tropical inbreds (summarized by Brewbaker, Kim and Logrono in Hawaii Res. Series 62, 1989). All tested monogenes for rust tolerance (e.g., *Rpp9*) were ineffective in Hawaii.

Set M of recombinant inbreds (RILs) was created in Hawaii from a hybrid of two supersweet inbreds, Iowa's Ia453*sh2* and Hawaii's Hi38*c1bt*, each a significant parent for commercial hybrids. The temperate inbred was highly susceptible while the tropical inbred showed an intermediate level of tolerance that minimized yield losses to sweet corn producers in Hawaii. The parents and 55 RILs (six generations selfed) were planted in a

three-rep RCB at Waimanalo Research Station (sea level) in the winter of 2003. The RILs from the very poorly adapted Iowa inbred were often very poor in vigor, intolerant of the low-light winter conditions and the high incidence of fusarium rots in this season.

Rust was scored on an empirical scale of 1 (highly tolerant) to 9 (highly susceptible), rating the entire plant. The parents averaged 3.1±1.58 (Hi38*c1bt*) and 5.3±2.09 (Ia453*sh2*). The 55 RILs ranged from 2.0 to 7.0 (Fig. 1), with an average of 4.45 and a distribution skewed toward susceptibility. We reported similar data (So, Ji and Brewbaker, MNL 77:32-33) for a generation mean analysis of a cross of Hi38-71, sister inbred of Hi38*c1*, with a susceptible field corn inbred, G24. Their rust scores (3.2 for Hi38-71, 6.9 for G24) were generally similar to those seen here. In that study the heterotic vigor of segregating progenies tended to bias the rust-readings toward resistance (F1 average was 2.9, F2 average was 4.2), and estimated gene number was 2.8. In contrast, the generally weak inbreds in the current study tended to be scored with bias toward susceptibility. It is possible to view the present data as two groups of inbreds, one tolerant (with disease scores lower than or equal to 4.4) and the other susceptible (with disease scores higher than 4.4). The distribution of RILs fitted a single-gene model tested by the normal frequency curve method (Moon, Maydica 44:301, 1999), but a digenic model appears more likely.

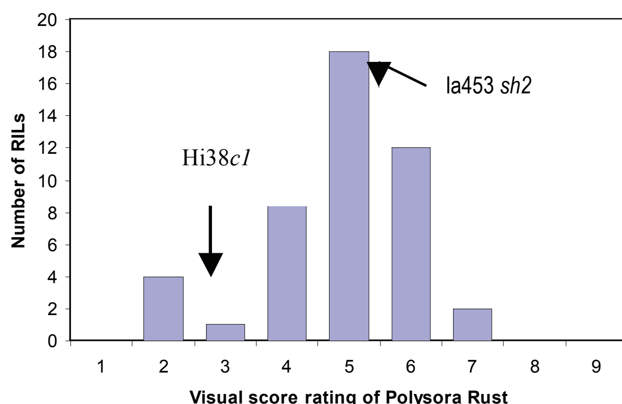

Figure 1. Frequency distribution of resistance to southern corn rust on RIL set M.

Inbreds such as parent Hi38*c1* showed many lesions on lower leaves, while leaves above the ear were often quite clean of rust. This tolerance has been described as “mature-plant resistance” or “late-rusting”. Careful digital estimates of lesion areas on a major leaf (e.g., subtending the ear) would probably distinguish genotypes more clearly, and the evaluation of vigorous testcrosses might best lead to their convincing distinction. In practice the tolerance of our “tolerant” RILs is adequate for production under severe epiphytotics in Hawaii.

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Glycolytic enzyme activity level in maize roots under low temperatures

--Sokolova, MG; Akimova, GP; Nechaeva, LV

Glycolysis, as the best studied respiration enzyme system, was selected for the present investigation of metabolic regulation mechanisms in the cell at lower temperatures. Potential activity of three major enzymes of glycolysis was identified: hexokinase (HK), pyruvate kinase (PK) and phosphofructokinase (PFK).

Growing cells of two maize varieties with contrasting cold resistance were used for the investigation. 48-hour-old seedlings of Omskaya 2 resistant variety and Uzbekskaya tooth-like non-resistant variety with zones marked 0-2 and 2-4 mm from the root tip were placed at temperatures of 27⁰C and 10⁰C (MNL 76:35-36, 2002). Cells of the 2-4 mm zone terminated extension at 27⁰C in 6 and 8 hours, and at 10⁰C in 48 and 96 hours in Omskaya 2 and Uzbekskaya tooth-like, respectively. There were also cells identified, which started extension within the time specified.

At 27⁰C, extension of maize seedling root cells of both varieties was accompanied by an increase of activity of all glycolytic enzymes under study. Nevertheless, the proportion of key enzymes in these varieties differed (Table 1). In the cells of the Omskaya 2 variety PK was more active than HK, with PFK being the least active enzyme. In the cells of the Uzbekskaya tooth-like variety starting extension, the following proportion was observed: HK>PK>PFK; in the cells which have completed extension the propertion PK>HK>PFK was observed. Increase of glycolytic element activity in the extending cells is associated with new formation of enzyme proteins, which is faster than their disintegration.

Table 1. Activity of key glycolytic enzymes in root cells of maize seedlings under low temperature, nmol of substrate/min per 10^6 cells.

Variety	Root growth zones	HK		PK		PFK	
		27⁰C control	10⁰C test	27⁰C control	10⁰C test	27⁰C control	10⁰C test
Omskaya 2	Extension start	25.8±1.0	11.8±0.5	36.4±1.9	21.1±0.9	16.5±0.5	5.3±0.1
	Extension termination	31.0±2.2	33.4±2.3	44.0±2.8	46.4±2.8	30.0±1.9	30.2±2.2
Uzbekskaya tooth-like	Extension start	27.8±2.3	48.6±3.2	16.4±0.7	47.6±3.1	12.2±0.6	18.8±0.9
	Extension termination	35.4±2.5	61.4±4.5	98.8±7.1	104.8±7.9	46.5±3.1	28.3±1.8

At 10⁰C, in cells of the Omskaya 2 variety that had started extension, the level of potential activity of glycolytic enzymes decreased significantly. The cells that had finished extension demonstrated the same activity of enzymes in control and test.

The non-resistant Uzbekskaya tooth-like variety demonstrated a reverse regularity: cell transfer to extension at 10⁰C was accompanied by the activity of the enzymes studied.

Thus, growth of maize root cells at low temperature is associated with differentiated effects in the activity of key glycolytic kinases depending on the cell growth stage and the resistance of the variety: root tip cells of the cold-resistant variety are characterized by the reduction of the speed of glucose destruction along the glycolytic pathway, with the traffic ability of the hexomonophosphate pathway increasing as shown before (Rodchenko, Maricheva, Akimova, 1988). This provides the cell with substrates

for multiple syntheses and increases metabolism resistance to stress impact. Extending cells of the non-resistant variety demonstrate a high intensity of exchange, which apparently exhausts the energy resources of the cell. Further cooling kills the plant.

Organic acids in maize seedling root cells growing at normal lower temperatures

--Akimova, GP; Sokolova, MG; Maricheva, EA

Organic acids, their formation and transformation play an important role in plant life cycles. Organic acids grow in number in plant cells under stress factors, including low temperatures. Accumulation of organic acids appears in the course of cell transfer from division to extension and differentiation.

We made an attempt to reveal probable differences in the content of organic acids in low temperature resistant plant varieties and non-resistant ones, and to show a connection between these differences and a change in speed of seedling root cell growth under hypothermia.

Organic acids were identified in the growth zones (beginning and end of cell extension) of maize seedlings of the resistant variety Omskaya 2 and the non-resistant variety Uzbekskaya tooth-like. The samples were placed in liquid nitrogen, then extracted by ethanol. Alcohol was evaporated, water fraction was cleaned by chloroform and sequentially passed through the columns with Daueks 50 and Daueks 1. Elution was conducted by 16N formic acid. Eluate was dried in a lyophilic kiln and prepared for gas liquid chromatography (GLC).

GLC analysis demonstrated peaks of malonate, succinate, fumarate, citrate, and malate. Peaks on chromatograms from growth zones of seedling roots of the Uzbekskaya tooth-like variety grown at 10^0C were higher than those of seedlings grown at 27^0C. Omskaya 2 manifested no increase of organic acid content.

Content of dicarbonate acids (malic and oxaloacetic) was determined in the growth zones of maize seedling roots based on 1 average cell (Table 1).

Table 1. Impact of lower temperature on the content of malic and oxaloacetic acids in the cells of maize seedling roots.

Root growth zones	Variant	Content, g x 10^{-12} per cell	
		malate	oxaloacetate
Omskaya 2			
Extension start	Control 27^0C, 6 h	8.84±0.51	0.89±0.06
	Test 10^0C, 48 h	9.75±0.62	0.90±0.07
Extension termination	Control 27^0C, 6 h	8.38±0.49	1.16±0.08
	Test 10^0C, 48 h	8.65±0.50	1.48±0.09
Uzbekskaya tooth-like			
Extension start	Control 27^0C, 8 h	18.63±0.09	4.99±0.02
	Test 10^0C, 96 h	30.80±1.91	5.97±0.03
Extension termination	Control 27^0C, 8 h	45.47±2.52	2.11±0.01
	Test 10^0C, 96 h	48.48±3.30	2.50±0.01

In cells of the non-resistant Uzbekskaya tooth-like variety that start extension at 10^0C, malate content is 1.7 times higher than the metabolite level in the cells extending at 27^0C. Content of oxaloacetate in the cells of cooled roots is insignificant. Temperature decrease did not considerably affect the number of these metabolites in the resistant variety.

Therefore, increase of organic acid content under hypothermia takes place only in cold-sensitive maize varieties, and is most pronounced in the cells that start visible growth at 10^0C. The latter presumably contributes to the growth of these cells' volume, which was described earlier (MNL 79:17, 2005), as well as to the decrease of cytoplasm pH, one of the plant responses to temperature change.

The study of foreign DNA's association with the main mitochondrial chromosome using isolated mitochondria

--Nepomnyaschih, DV; Dietrich, A; Konstantinov, YM

It was shown previously (MNL 64:67-68, 1990, EMBO J. 22:1245-1254, 2003; MNL 78:20, 2004) that isolated maize and potato mitochondria can efficiently uptake extramitochondrial DNA. Little is known at the moment about the possibility of imported DNA's association and/or integration into the main mitochondrial DNA. To study the specificity of association between foreign DNA imported into isolated mitochondria and mitochondrial DNA, we used different genetic constructs with or without regions of homology to the main mitochondrial chromosome. For this purpose we prepared 4 types of constructs to be used as substrates for DNA import into mitochondria: (1) the *DR-Zm/gfp* construct contains regions identical to the maize mitochondrial genome, (2) the *nad2St/gfp* construct contains regions with partial homology to the maize mitochondrial genome and identical to the potato mitochondrial genome, (3) the *pBs-KS* construction (a linear form of the plasmid) has no homology to mitochondrial DNA, (4) the *gfp* construct (GFP gene fragment with ca 400 bp size) used in the *DR-Zm/gfp* and *nad2St/gfp* constructs has no homology to mitochondrial DNA.

In this note, we report some evidence about the association of foreign DNA, which penetrates into maize mitochondria through the DNA import mechanism with the main mitochondrial chromosome.

Maize mitochondria were isolated from 4-day-old etiolated seedlings of hybrid VIR42 MV by the standard method of differential centrifugation. The substrate DNA used for mitochondrial import assays were the constructs *DR-Zm/gfp*, *nad2St/gfp*, linear *pBluescript-KS*+ and a fragment of the *gfp* (Green Fluorescent Protein) gene. To obtain the radioactive linear fragment, 50 ng of unlabeled PCR product and corresponding primers were used for a single PCR cycle in which a 10 min elongation in an unlabeled dCTP-deprived reaction medium containing 100 mCi of [α-32 P] dCTP (3000 Ci/mmole) per 50 ml was followed by the addition of 0.2 mM unlabeled dCTP and a further 5 min elongation. Standard mitochondrial import of DNA was carried out in 40 mM potassium phosphate and 0.4 M sucrose pH 7.0 (import buffer). The samples containing 5-10 ng of ^{32}P-labeled DNA and an amount of purified mitochondria corresponding to 200 μg of proteins were incubated at 25°C for 45 min under mild shaking. Then, mitochondria were pelleted and resuspended in buffer containing 330 mM sucrose, 90 mM KCl, 10 mM MgCl2 , 12 mM tricine, 5 mM KH_2PO_4, 1.2 mM EGTA, 2 mM DTT, 2 mM ADP, 10 mM sodium succinate, and 0.15 mM of each dNTP. After incubation at 25°C for 60 min under mild shaking, mitochondria were pelleted and the final pellets were extracted with one volume of 10 mM Tris-HCl, 1 mM EDTA, 1% (w/v) SDS pH 7.5 and one volume of phenol. The nucleic acids recovered in the aqueous phase were ethanol-precipitated, fractionated by electrophoresis on a 1% (w/v) agarose gel and transferred onto a nylon membrane (Hybond N+, Amersham Biosciences) for autoradiography.

We showed (Fig. 1) that the existence of species-specific sequences in the *DR-Zm/gfp* construct is the main requirement for imported DNA to be associated and/or integrated into the main mitochondrial DNA. We need additional evidence now to be sure that foreign sequences associated with the main mitochondrial DNA are integrated into the mitochondrial chromosome.

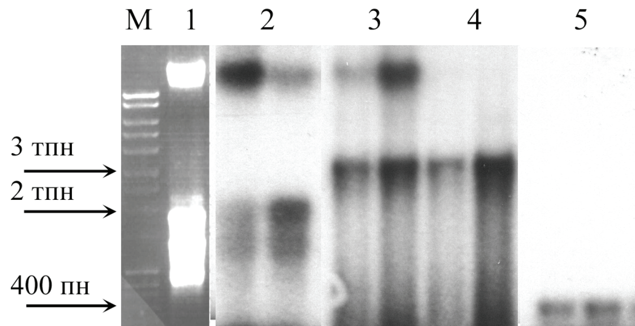

Figure 1. Study of the association of DNA imported into isolated maize mitochondria with high molecular weight mitochondrial DNA. 1) High molecular weight DNA of non-treated mitochondria (ethidium bromide staining); 2) the *DR-Zm/gfp* construct (electrophoresis in denaturation conditions); 3) the *DR-Zm/gfp* construct; 4) the *nad2St/gfp* construct; 5) the *gfp* construct.

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Inhibitory analysis of protein phosphorylation/dephosphorylation in mitochondria

--Subota, IY; Arziev, AS; Tarasenko, VI; Konstantinov, YM

Phosphorylation/dephosphorylation of amino acid residues is the most common posttranslational modification of proteins which can alter enzyme activity, half-life of the protein, etc. Protein kinases and protein phosphatases which perform this reactions probably function as tightly associated complexes regulated by feedback mechanism. The excess of protein kinase leads to an activation of protein phosphatase which in turn dephosphorylates and inactivates protein kinase.

In spite of the importance of this modification, little is known about the role of protein phosphorylation in plant mitochondria. In this work, we studied the influence of redox conditions on the level of phosphorylation of individual mitochondrial proteins as a possible mechanism of redox regulation of mitochondrial processes, including gene expression.

The mitochondria were isolated from 3-day-old etiolated maize seedlings (hybrid VIR42MV) by the standard method of differential centrifugation. Protein phosphorylation assays were carried out according to Struglics et al. (FEBS Lett. 475:213-217, 2000) with the use of [γ^{32}P]ATP (specific radioactivity was 6000 Ci/mmol).
Incubation of mitochondria with [γ^{32}P]ATP resulted in phosphorylation of 8 proteins as judged by SDS-PAGE and autoradiography. Potassium ferricyanide used as an oxidizing agent decreased ^{32}P incorporation into all of these proteins (Fig. 1). Treatment with the reducing agent sodium dithionite reduced specifically the labeling of the 62 kDa protein. When the physiological redox agent glutathione was used, we also observed some alterations in protein phosphorylation (Fig. 1). The addition of oxidized glutathione

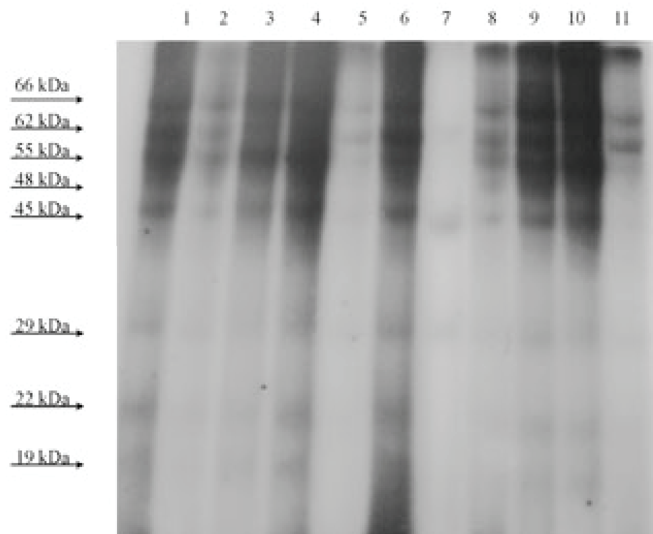

Figure 1. The effect of redox agents and protein kinases and protein phosphatase inhibitors on mitochondrial phosphorylation. Lane 1, no agents; lane 2, 5 mM potassium ferricyanide; lane 3, 5 mM sodium dithionite; lane 4, 5 mM GSH; lane 5, 10 mM GSSG; lane 6, 200 nM staurosporine; lane 7, 40 mM NaF; lane 8, ferricyanide+NaF; lane 9, ferricyanide+staurosporine; lane 10, dithionite+NaF; lane 11, dithionite+staurosporine.

resulted in a substantial decrease in protein phosphorylation. When comparing the inhibitory effects of two oxidising agents on protein phosphorylation, GSSG has been demonstrated to be more effective than potassium ferricyanide. GSH used as a reducing agent inhibited ^{32}P incorporation into two proteins of 62 and 55 kDa.

In our experiments, 200 nM staurosporine reduced the level of phosphorylation of the 55 kDa protein. Simultaneous addition of redox agents and inhibitors of protein kinases (staurosporine) and protein phosphatases (sodium fluoride) modulated the activity of protein phosphorylation. When potassium ferricyanide was added together with sodium fluoride, the rate of protein phosphorylation increased in comparison with the effects of these agents alone.

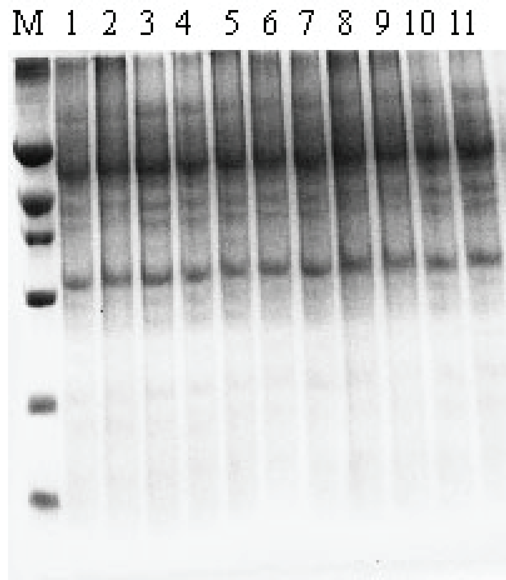

Figure 2. Coomassie-stained gel identical to that used in Figure 1. M-ladder; lanes 1 – 11 are the same as in Figure 1.

The Coomassie-stained gel showed equal loading of all lanes (Fig. 2). A comparison with the autoradiograph demonstrated that the polypeptide and phosphoprotein patterns were quite different.

Thus, on the basis of the results obtained, we may conclude that inhibition of both protein kinases and protein phosphatases in different redox conditions clearly affects the protein phosphorylation activity in mitochondria. These data permit a supposition about the existence of redox sensitive protein kinases and protein phosphatases in plant mitochondria which modify the effects of redox conditions on protein phosphorylation in organello.

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Identification of cDNA for a new chloroplast Cu/Zn superoxide dismutase in maize

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Mitochondria and chloroplasts are major sources of reactive oxygen species (ROS) in plant cells. To minimize the damaging effects of ROS, plants have evolved both enzymatic and nonenzymatic antioxidant defense systems. Superoxide dismutases (SODs, EC 1.15.1.1) are pivotal enzymes of the enzymatic defense system that are specifically compartmentalized in various cellular organelles, including mitochondria and chloroplasts. In maize mitochondria, four different MnSOD isozymes encoded by distinct nuclear genes have been identified (Zhu and Scandalios, 1993). Each member of this small maize MnSOD multigene family is differentially regulated during development in response to plant growth regulator abcisic acid and high osmoticum (Zhu and Scandalios, 1993). In contrast to mitochondria, only one nuclear-encoded Cu/ZnSOD has been found in chloroplasts (Kernodle and Scandalios, 2001). The method of accurate regulation of superoxide and other ROS levels regulation in this organelle still needs further investigation. Previously, we reported the identification of novel cDNA for the chloroplast FeSOD gene (Katyshev et al., 2005). In this report, we present data on characterization of partial cDNA corresponding to a novel maize chloroplast Cu/ZnSOD gene which differs from that reported by Kernodle and Scandalios (Kernodle and Scandalios, 2001).

Total RNA isolation from 3-day-old etiolated hybrid maize VIR46MV seedlings was performed by QIAGEN RNeasy Mini Kit according to the manufacturer's instructions. cDNA synthesis was carried out using the Promega Universal RiboClone cDNA Synthesis System. In order to amplify 3'-ends of probable cDNA corresponding to the chloroplast Cu/ZnSOD gene, 3'-RACE (rapid amplification of cDNA 3'-ends) experiments using the previously described primers chcsc1 and 3UTR (Katyshev et al., 2006) were performed. As a result, approximately 400 bp-long RT-PCR product formation was observed. This cDNA fragment was eluted from agarose gel, blunted by Klenov fragment of *E. coli* DNA polymerase and ligated in pBlueScript KS(+) plasmid (Fermentas, Lithuania) by *Eco*RV site. After transformation, several positive XL1-Blue *E. coli* clones were chosen for plasmid DNA isolation and insertion sequencing using universal M13/pUC primers (Fermentas, Lithuania). The analysis of cloned sequences resulted in identification of two cDNA clones (CZ6 and CZ8) containing 3'-ends of novel maize Cu/ZnSOD cDNA (the submission of the sequences to EMBL/GenBank databases is in progress).

A search for similar nucleotide sequences by BLAST services at the PlantGDB server (http://www.plantgdb.org/PlantGDB-cgi/blast/PlantGDBblast) found several highly similar maize EST sequences (e.g., EMBL/GenBank acc. numbers CF042260, CF008113, CO443266, DN222176, DR795998, DR795999). Multiple alignment of the EST sequences found and reconstruction of the chimerical consensus sequence for the corresponding full-length cDNA, allowed us to get a probable translated sequence of novel maize Cu/ZnSOD cDNA. To predict subcellular localization of translated protein sequence, the Internet resources available at the http://www.expasy.org/ molecular biology tools server were used: a) *the Predotar program at the* http://www.inra.fr/predotar/; b) the TargetP V1.1 program (Emanuelsson et al., 2000) at the http://www.cbs.dtu.dk/services/TargetP/; c) the WoLFPSORT program (Horton et al., unpublished)) at the http://wolfpsort.seq.cbrc.jp/; and d) the Chlorop v1.1 program (Emanuelsson et al., 1999) at the http://www.cbs.dtu.dk/services/ChloroP/. For comparison, subcellular localization of SOD1 protein (Kernodle and Scandalios, 2001) also was checked. The results of subcellular localization prediction are presented in Table 1 (the amino acid sequence corresponding to the novel *Zea mays* Cu/ZnSOD is referred to as SOD1.2).

Table 1. Probabilities of chloroplast and nonchloroplast localization of two Cu/ZnSOD (SOD1 and SOD1.2) proteins predicted by different programs.

Name of program	Probability of chloroplast localization		Probability of localization in other cellular compartments	
	SOD1	SOD1.2	SOD1	SOD1.2
Predotar v. 1.03	0.01	0.93	0.18 – mitochondrion 0.01 – ER 0.81 – other	0.16 – mitochondrion 0.03 – ER 0.06 – other
Targetp v1.1	0.098	0.349	0.116 – mitochondrion 0.307 - extracellular 0.403 – other	0.372 - mitochondrion 0.196 - extracellular 0.009 – other
WoLFPSORT	-	0.839	0.595 – cytosol 0.118 - nucleus	0.18 – mitochondrion
Chlorop v1.1	0.463	0.551	-	-

The results of in silico subcellular localization prediction demonstrate that the SOD1.2 protein could be referred to as chloroplast protein even to a larger extent than the SOD1 enzyme. In fact, results of multiple alignment of plant Cu/ZnSOD cDNA sequences not presented in this article provide further support for such a conclusion.

Briefly, in this report we provide data demonstrating the existence of the novel Cu/ZnSOD gene cDNA in maize seedlings that encode, as was predicted in silico, chloroplast enzymes. These data led us to suggest that in the maize chloroplast several superoxide dismutase enzymes (two Cu/ZnSOD and one FeSOD) function together and are encoded by distinct genes similar to MnSODs in mitochondria. Such results are not surprising since both of these organelles are the major sources of cytotoxic superoxide radicals in plant cells.

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Using double haploid lines for quantitative trait analysis

--Mihailov, ME; Chernov, AA

Forty-five double haploid (DH) lines of maize derived from a MK01 x A619 hybrid were tested for quantitative traits in 2003-2005. Forty lines were tested in all three years, 5 lines in one or two years. These lines were obtained by colchicine doubling haploids that were produced by pollinating the F1 with Moldavian Haploid Inducer (MHI). Therefore, the genome of the DH lines is the doubed genome of female gametes of the F1. Genes of both parents are presented in various combinations in the set of DH lines except for heterozygotes.

In accordance with an additive-dominant model, the distribution of the DH lines for a quantitative trait would be symmetric around midparent values (the mean of MK01 and A619): half of the DH lines would be above midparent values and the other half would be below. In reality, some traits (productivity, ear diameter, number of kernels, number of ears, time of flowering and maturity) show stable asymmetry (see Table 1). This suggests the important role of non-allelic interactions in the genetic control of these traits.

Table 1. Distribution of DH lines relative to midparent.

	Distribution ratio of the DH lines (above : below)		
Trait	2003	2004	2005
Time of tassel flowering	4:37 **	9:35 ***	6:38 ***
Tine of silk flowering	6:34 **	9:35 ***	5:39 ***
Anthesis-silking interval	24:16	19:25	19:25
Flowering-maturity interval	15:24	14:30 *	30:14 *
Time of maturity	10:30 **	9:35 ***	14:30 *
Height of plant	7:34 ***	7:37 ***	20:24
Length of steam	5:36 ***	8:36 ***	21:23
Length of tassel	21:20	22:22	13:31*
Diameter of steam	15:26	21:23	14:30 *
Number of nodes	7:34***	13:31***	21:23
Number of ears	12:29 *	14:30 *	11:33 **
Weight of cob	15:26	19:25	19:25
Length of ear	10:31 **	18:26	10:34 ***
Diameter of ear	3:38 ***	4:39 ***	6:38 ***
Number of kernel rows	17:24	15:28	10:33 ***
Number of kernels	4:37 ***	6:38 ***	15:29 *
Weight of 1000 kernels	23:17	19:24	24:20
Productivity of first ear	3:38 ***	6:38 ***	14:30 *
Productivity of second ear	6:35 ***	12:32 **	10:34 ***
Total productivity	2:39 ***	6:38 ***	14:30 *

Comment. Disagreement with 1:1 ratio *P<0.05, **P<0.01, ***P<0.001.

Apparently, genetic interactions in some traits (weight of cob, weight of 1000 kernels, anthesis-silking interval, length of tassel, diameter of steam) are mainly additive and dominant. Distribution of other traits is intermediate or unstable.

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Evaluation of incidence and severity of *Puccinia sorghi* and other diseases in the inbreds of Andino-Patagonico in Argentina

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During the 2004/05 growing seasons, Andinas-Patagonicas inbreds were evaluated for response to the attack of the fungal pathogen responsible for maize rust and other leaf diseases. The selected lines were from the Institute Fitotécnico de Santa Catalina: SC1, SC3, SC4, SC6, SC7, and SC9 were harvested from the west Patagonica zone in Argentina and X Region in Chile. Also included were one early line, F2 (France), and two lines of average cycle, B73 and Mo17 (USA). The materials were sown on 2 dates, with the second 20 days after the first. The severity of attack was determined for three leaves: Leaf 1: under ear, Leaf 2: ear leaf and Leaf 3: above the ear. Plants with one pustule or less were considered to be tolerant (t).

Most lines present a high incidence (>70 %) of *P. sorghi* for both plantings, with the exception of SC1 (46%) (Table 1). Lines SC4 and SC6 exhibited reduced severity (Table 2). We also observed the presence of other diseases, such as leaf spot caused by *Helminthosporium sp.* and also corn smut, caused by *U. maydis.* These were of lesser severity. In the majority of cases, the incidence was more obvious for the first planting (Table 2).

Table 1. Frequency of *Puccinia sorghi* infection.

Lines	1st Planting % infected	2nd Planting % infected
SC1	46	100
SC3	87.7	100
SC4	96.9	88.4
SC6	76.4	86
SC7	85.1	81
SC9	95.2	85.6
F2	76.9	100
B73	47.5	89.4
Mo17	100	47.9

Table 2. Severity of *Puccinia sorghi* infection in leaves.

1st Planting	*P. sorghi* leaf 1 %	*P. sorghi* leaf 2 %	*P. sorghi* leaf 3 %	Other diseases
SC1	0-t	0-t	0-t	*Helminthosporium spp*
SC3	0-1	0-1	0-1	*Helminthosporium spp*
SC4	0-1	0-1	0-1	*U. maydis, Helminthosporium spp*
SC6	0-1	0-1	0-1	*Helminthosporium spp*
SC7	0-1	0-1	0-1	*U. maydis, Helminthosporium spp*
SC9	0-5	0-5	0-5	*Helminthosporium spp*
F2	0-10	0-15	0-10	------
B73	0-10	0-10	0-10	------
Mo17	0-15	0-10	0-10	*U. maydis*
2nd Planting	*P. sorghi* leaf 1 %	*P. sorghi* leaf 2 %	*P. sorghi* leaf 3 %	Other diseases
SC1	0-20	t-30	t-30	------
SC3	0-5	t-10	t-10	*Helminthosporium spp*
SC4	0-t	0-1	0-1	------
SC6	0-t	0-1	0-1	------
SC7	0-1	0-1	0-1	*U. maydis*
SC9	0-1	t-5	0-1	------
F2	t-5	t-5	t-5	------
B73	0-t	0-t	0-t	------
Mo17	0-1	0-1	0-1	------

Scoring: None ------ ; average: 0-30% ; t : tolerant.

The lines SC4 and SC6 were selected to continue improving tolerance to maize rust and other diseases in the materials employed in this breeding.

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Chemical composition of F2 kernels from high quality maize single crosses

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Since the old days, plant breeding has contributed to the increase in content of nutritional elements, as well as to improving the efficiency of their production. Nevertheless, the conscious improvement of nutritional elements may be considered a more recent goal in plant breeding. In 1990, a maize quality breeding program was initiated at the CIGEN, which is located in Llavallol in the province of Buenos Aires, Argentina (22 m.a.s., 34°48´S; 58° 31´W), aimed at developing and selecting high oil, high protein, high starch and high quality protein inbreds for further development of quality single crosses. During the first stage of the program, several maize inbreds were developed, tested and selected following the classic methodology. These inbreds carry single recessive genes (*wx, o2, o5, O9, O11, sh4*) or may be double recessive (*wxsh4; wxo2*). Moreover, high oil inbreds were also selected during this phase. In the following stage of breeding, single-cross hybrids were obtained and tested in several complete randomized block design field trials with three replicates conducted in Llavallol. The experimental unit was a 5.5 m long row. The experimental hybrids tested were named from CIG 1 to CIG 104. During the last three years (2003, 2004 and 2005) protein, starch and oil content, as well as kernel density, were evaluated in F2 kernels from a total of 104 single crosses by near infrared reflectance (NIR) using a Tekator Infrated 1227 device. The field trials were kept in isolation from other maize to prevent the influence of foreign pollen on oil content, and protein and starch quality.

In 2003, all hybrids were evaluated in field trials and the results are presented in Table 1. Later, in 2004 and 2005, only eighty and sixty-three single crosses were tested respectively (see Tables 2 and 3). Certainly, the greatest variation among genotypes is for oil and protein content, as the coefficient of variation obtained for starch content and kernel density is truly very low. Only seven hybrids were studied during the three growing seasons. Forty-eight single-cross hybrids were evaluated for two years and the remaining forty-nine crosses were only studied for one year.

Table 1. Results for 104 hybrids evaluated during the first year of trials (2003).

	avg. ± s.	variance	c.v.%	min.	max.
protein	9.97 ± 0.92	0.85	9.23	8.7	12.1
oil	5.08 ± 0.40	0.16	7.87	4.28	6
starch	71.6 ± 1.13	1.28	1.58	69.1	74.1
density	1.27± 0.03	0.17	2.36	1.21	1.31

Table 2. Results for 80 hybrids evaluated during the second year of trials (2004).

	avg. ± s.	variance	c.v.%	min.	max.
protein	10.5 ± 0.95	0.9	9.09	8.4	12.9
oil	5.4 ± 0.48	0.23	8.89	4.48	6.04
starch	70.4 ± 0.99	0.98	1.4	68	72.8
density	1.29 ± 0.01	0.0001	0.78	1.25	1.31

Table 3. Results for 63 hybrids evaluated during the third year of trials (2005).

	avg. ± s.	variance	c.v.%	min.	max.
protein	11.45 ± 0.78	0.61	6.81	9.3	13.1
oil	5.36 ± 0.5	0.25	9.33	4.49	6.89
starch	70.5 ± 1.21	1.46	1.72	68	73.8
density	1.27 ± 0.04	0.002	3.15	1.07	1.32

The average protein content in the whole kernel does not differ significantly among five hybrids studied from 2003 to 2005: CIG6, CIG9, CIG42, CIG58 and CIG59 (ANOVA, $F_{6\text{-}12}$: 0.40; p: 0.87). Nevertheless, highly significant differences in the protein content of each single-cross were detected among years (ANOVA, $F_{2\text{-}12}$: 4.35; p: 0.04), and two different groups were detected through the least significant differences test (LSD, D: 0.937; t_{12}: 2.18; S_x: 0.430; p: 0.05). The differences found in relation to oil content among the seven genotypes evaluated for three consecutive years (ANOVA, $F_{6\text{-}12}$: 2.45; p: 0.09) made it possible to also differentiate two groups (LSD, D: 0.562; t_{12}: 2.18; S_x: 0.258; p: 0.05). No significant differences among years for the same genotype were found in relation to oil content (LSD, D: 0.368; t_{12}: 2.18; S_x: 0.169; p: 0.05).

In relation to the 48 single crosses evaluated for two years, the results showed highly significant differences among genotypes for protein content (ANOVA, $F_{47\text{-}47}$: 1.88; p: 0.016) and also for oil content (ANOVA, $F_{47\text{-}47}$: 1.30; p: 0.18). These hybrids may be divided into nine groups in relation to their protein content (LSD, D: 1.5602; t_{47}: 2.012; S_x: 0.776; p: 0.05) or into seven groups if oil content is considered (LSD, D: 1.03; t_{47}: 2.012; S_x: 0.513; p: 0.05). Protein content differs very significantly through the two years of evaluation (ANOVA, $F_{1\text{-}94}$: 17.37; p: 0.000) and two groups may be distinguished (LSD, D: 0.319; t_{47}: 2.012; p: 0.05). No differences were found in relation to oil content between years (ANOVA, $F_{1\text{-}47}$: 0.11; p: 0.74). No variation (c.v.: 0%) was observed in the oil content of hybrids CIG11, CIG22, CIG38 AND CIG53 through the period evaluated. Therefore, these single crosses containing 4.8% to 6.0% oil may be considered to be very stable for the trait. No significant correlations were found between oil and protein content (r: 0.12). Nevertheless, significant correlations exist between: oil-starch (r: -0.24; p: 0.05); starch-protein (r: -0.71; p: 0.05); oil-density (r: -0.35; p: 0.01) and starch-density (r: -0.53; p: 0.05). The protein content observed in the F2 kernels of the single-cross hybrids studied (e.g.: 13.1% in CIG21, see Tables 1 to 3) is certainly high in relation to the average content of the commercial hybrids commonly grown in Argentina.

The results obtained to this point, reinforced by a significant variation among years for protein content in most of the genotypes, strengthen the already known fact that *environment* and *genotype x environment interaction* are extremely important in the determination of this trait. Nevertheless, in spite of this fact, a high level of protein content in F2 kernels resulted from using a very high protein content inbred as female parent. Whether or not most of the single-cross hybrids tested have a high protein content (10.5% to 13.1%) and also a high starch content (70% to 72%), all of them have a high oil content (≥ 4.4%).

The great quality of these single-cross hybrids, accompanied by their performance and phytosanitary behaviour, will facilitate their use for animal feed and for obtaining high value-added consumer goods in the near future.

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A defect of maltase enzyme activity in the *sugary enhancer (se)* mutant
--Pan, D

The sugary (*su)* genotype, which is commonly used as sweet corn, differs from other vegetable corns by its ability to produce large amounts of phytoglycogen, an important factor in the texture of an edible product. However, the *sugary* gene does not produce as high a level of endosperm sugars as *shrunken2* or *brittle1* or *2.* There was another line of sugary corn discovered by A. M. Rhodes named *sugary enhancer (se)* that possesses certain characteristics differing markedly from normal sugary corn. The sugar content of the endosperm is comparable to *sh2* without a loss of phytoglycogen. Later, it was found that *sugary enhancer (se)* has considerably higher content of maltose as compared to any other sugary corns. Here we report that the accumulated higher content of maltose in *sugary enghancer (se)* is due to a defect of maltase enzyme activity. Maltase catalyzes the following enzyme reaction:

Thus, the *sugary enghancer gene (se)* as recommended by Rhodes could code for maltase protein as its gene product.

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Heritability and correlation studies in sweet corn for quality traits, field emergence and grain yield
--Kumari, J; Gadag, RN; Jha, GK

Ten sweet corn and seven field corn genotypes were studied for estimating components of genetic variance and combining ability for ear-related and biochemical traits using diallel and "line-X-tester" mating designs. The study was carried out at the Division of Genetics and Division of Environmental Science, Indian Agricultural Research Institute, during 2003-2004. Diallel crosses were made among six inbred lines, including three each of sugary and shrunken genotypes, while in later crosses seven field corn inbreds were crossed as female lines with four sweet corn inbreds as tester parents. The details regarding pedigree, source of origin and endosperm mutants are given in Table 1.

Analyses of variance by diallel shows the mean sum of squares is highly significant for all nine traits except reducing sugar (Table 2). In the line-X-tester analysis (Table 3), parents as a whole are significant at 1% for all characters, while the partitioned source of variance for female parents indicated significance for starch, carbohydrate, grain weight, field emergence and TSS. This meant that field corn lines have significant variability for these traits while significant for sugar components and phytoglycogen. As far as male parents or sugary lines are concerned, these were significant for all sugar components, phytoglycogen and total carbohydrate components. As it is well known that field and sweet corn populations are genotypically and phenotypically different, the female vs. male source of variation is significant for all traits. Similarly, hybrid and parent vs. hybrid mean sum of squares are significant for most of the traits. This suggests the utilization of non-sweet germplasm in the improvement of sugary genotypes through hybridization and introgression, followed by backcrossing for characters such as field emergence, plant stand and yield.

Table 1. Pedigree and source of origin of inbred lines.

Inbreds (Code #)	Pedigree	Source population	Endosperm mutation
DMB321	IPA40-f-17-1-1-4-1-1-1-f	AD-609	Normal
DMB322	TCA-22-3-1-1-2-f-#-f-1-1	A-64	Normal
DMB323	IPA-1-f-16-2-#-f-1	A-64	Normal
DMB324	IPA-34-5-f-1-1	MDR-1	Normal
DMB325	PC2HS-31-f	PC2composite	Normal
DMB326	IPA-3-6-10-3-1-1-1-2-1	A-64	Normal
DMB327	TCA-21-1-b-1-1-1-3-1	AD-609	Normal
SCI301	SCMD90 (01R)-2-1-3-1	Madhuri	*su*
SCI302	SCMD90 (01R)-3-1-2-1	Madhuri	*su*
SCI303	SCMD90 (01R)- 4-2-1-2	Madhuri	*su*
SCI304	SCMD90 (01R)- 4-3-2-1	Madhuri	*su*
SCI305	SCMD90 (01R)-5-4-1-1	Madhuri	*su*
SCI306	SCPRHY85 (01R)-2-1-2-3	SOOK SH137	*sh*
SCI307	SCPRHY85 (01R)-6-3-1-2	SOOK SH137	*sh*
SCI308	SCPRHY85 (01R)-7-3-2-1	SOOK SH137	*sh*
SCI309	SCPRHY90 (01R)-2-2-1-2	SOOK SH138	*sh*
SCI310	SCPRHY90 (01R)-3-1-3-1	SOOK SH138	*sh*

The correlation coefficients were calculated to determine the degree of association of characters among the kernel quality components total soluble solids (TSS), grain yield and field emergence. Phenotypic correlations were computed using the formula given below. Pearson product-moment correlation coefficients were calculated using inbred line means from replicated trials of diallel and line-X-tester analysis using the SPSS 10.0 package.

$$r_p = \frac{Cov \quad XY_p}{\sqrt{X_p^2 \ \ x \ \ Y_p^2}}$$

Where,
r_p = Phenotypic correlation
Cov $(XY)_p$ = Phenotypic covariance between the characters *X* and *Y*
X^2_p and Y^2_p = Phenotypic variance of the characters *X* and *Y*, respectively
Phenotypic correlation coefficients were compared against '*r*' values at (n-2) d.f. at the probability levels of 0.05 and 0.01 to test their significance. The results of correlation analysis for the traits studied in this experiment are presented in Table 4.

The main objective associated with this study was to understand the relationship between field emergence, grain weight and kernel quality traits, including total soluble solids (TSS). The correlation analysis revealed that total sugar is positively correlated with reducing sugar and non-reducing sugar with a high level of significance ($p < 0.001$). Phytoglycogen and total soluble solids had significant correlation with total sugar, with p values equal to 0.011 and 0.007, respectively. However it had negative significant correlation with starch, carbohydrate, grain weight and field emergence. The same trend was exhibited by reducing sugar as well as non-reducing sugar. Starch concentration in dry mature kernels was most highly correlated with total carbohydrate (r = 0.78), followed by field emergence (r = 0.69) and grain weight (r = 0.52) while it was negatively correlated with all other characters. The phytoglycogen content had a highly negative correlation coefficient with

Table 2. Analysis of variance for diallel.

Source	d.f.	Mean sum of squares								
		Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Total Starch (%)	Phytoglycogen (%)	Total carbohydrate (%)	Grain weight	Field emergence	Total soluble solids (TSS)
Replication	2	0.48	0.087	0.54	22.01	0.47	29.09	30.96	44.92	0.29
Treatment	20	14.04**	0.34	11.22**	209.24**	89.87**	89.38**	9.68**	236.54**	3.13**
Error	40	1.68	1.58	1.65	22.06	0.50	26.07	3.78	14.17	1.11

* and ** indicate significance level at 1% and 5 % respectively.

Table 3. ANOVA for parents and hybrids (biochemical traits) in line-X-tester.

Source	d.f.	Mean sum of squares								
		Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Total Starch (%)	Phytoglycogen (%)	Total carbohydrate (%)	Grain weight	Field emergence (%)	Total soluble solids (TSS)
Replication	2	2.25	0.77	5.09	136.81	0.04	103.79	5.03	37.27	0.29
Parents	10	51.37**	2.18**	33.86**	516.53**	117.66**	132.07**	50.13 **	335.60**	3.13**
Females	6	0.61	0.16	0.29	120.24**	0.032	117.83**	29.20 **	407.19**	3.07**
Males	3	27.13**	0.35*	24.17**	10.54	165.21*	76.13*	16.33	141.60	1.14
Females vs. Males	1	428.67**	19.76**	264.35**	4412.31**	680.83**	385.35**	277.09 **	488.08**	9.44**
Hybrids	27	12.23**	0.97**	13.80**	240.10**	7.78**	198.37**	28.77 **	173.89**	0.76
Parents vs. hybrids	1	773.98**	37.18**	471.93**	378.42**	83.81**	0.64	5.37	8713.50**	117.87**
Error	76	2.10	0.09	2.15	31.60	0.37	27.57	6.53	79.61	1.11

* and ** indicate significance level at 1% and 5%, respectively.

Table 4. Correlation coefficients among kernel characteristics, yield and field emergence.

	TS	RS	NRS	ST	**PH**	TC	GW	FE	TSS
Total sugar (TS)	1.00	0.89	0.99**	-0.81**	0.43*	-0.54**	-0.49*	-0.28	0.46*
Reducing sugar (RS)		1.00	0.84**	-0.82**	0.61**	-0.47*	-0.64**	-0.34	0.50*
Non-reducing sugar (NRS)			1.00	-0.78**	0.37	-0.54**	-0.44*	-0.25	0.43*
Total starch (ST)				1.00	-0.67**	0.78**	0.51**	0.69**	-0.30
Phytoglycogen (PH)					1.00	-0.13	-0.67**	-0.28	0.19
Total Carbohydrate (TC)						1.00	0.94**	-0.05	-0.13
Grain Weight (GW)							1.00	0.54**	-0.35
Field Emergence (FE)								1.00	0.23
Total soluble solids (TSS)									1.00

* and ** indicate significance at 1% and 5%, respectively.

grain weight and starch content, whereas there was no significant correlation with field emergence and total soluble solids. Further, total carbohydrate exhibited negative but significant correlation with all sugar components and positive significant correlation with starch content. As far as grain weight is considered, there was significant correlation in a negative direction with all quality traits except starch and carbohydrate content. It also displayed a positive correlation with field emergence, but negative with TSS. Field emergence, the important aspect for sweet corn, was positively correlated with only two traits viz. starch content and grain weight. When the total soluble solids were studied at 20-22 days after pollination to observe the relationship between the above-mentioned characters, highly significant correlation was observed with sugar components.

The sugar components, in general, were highly correlated among themselves and negatively correlated with starch content, total carbohydrate, grain weight and field emergence. This kind of association was also observed by Churchill and Andrew (Crop Sci. 24:76-81, 1984) and Azanza et al. (Euphytica 87:7-18, 1996). The lower correlation of field emergence with high sugar level can be attributed to the fact that high sucrose concentration in the endosperm during kernel development interferes with the normal development of either the endosperm or the embryo, resulting in a decrease in the ability of the kernel to germinate and emerge in the field. Douglass et al. (Seed Sci. Tech. 21:433-445, 1993) also reported such a negative association of field emergence with sugar concentration and suggested that genotypes with greater sugar concentrations would display a more negative osmotic potential during seed hydration and during germination, and that the steep osmotic gradient can induce the rapid influx of water leading to membrane disruption and the leakage of the water-soluble fraction from the endosperm. Starch concentration in dry matured kernels is highly correlated with field emergence ($r = 0.69$, $p< 0.01$) and suggests that the carbohydrate reserve accumulated during kernel maturation plays an important role in field emergence. These reserves remain available to the embryo to be metabolized and used as an energy source for germination (Douglass et al., 1993). Total starch concentration was found to be highly correlated with kernel dry weight. This is expected since starch is the major contributor to the grain weight. As a consequence, grain weight is also positively correlated with field emergence ($r = 0.54$, $p<0.01$).

The unfavorable correlation coefficients between sugar content and grain weight suggested that in breeding programmes it is difficult to obtain high yielding sweet maize hybrids. A similar result was obtained by Has (MNL 77:74-75, 2003), and it is also clear from the present study of hybrid performances for these two traits. Furthermore, the positive association between total soluble solids and sugar components implied that estimation of TSS at 20 days after pollination could help in the evaluation and screening of a large number of genotypes for assessing sugar content. However the nonsweet germplasm has the potential to improve sugary genotypes for traits like field emergence, plant stand and yield.

Physical characteristics of different types of maize kernels

--Gadag, RN; Jha, SK; Singh, A

Maize is considered an important cereal crop serving as a staple food to a large population spread over parts of Africa, Asia, North and South America. It is the most diversified of all cereal grains in terms of its application. Maize is utilized for human food, industrial processing and as a feed ingredient for animal rations. Various types of maize, classified by characteristics of their kernel endosperm, have been developed, including field (flint and dent), flour, popcorn, sweet and waxy corns. These are found to exhibit a specific pattern of distribution of endosperm in respect to content and component of starches (hard and soft), which determines their end uses (Hallauer, A.R. (Ed), 'Speciality Corns,' SRC Press Inc., Boca Raton, USA, 1994. Pp 410). Kernels of these different types of maize can also be visually distinguished due to their characteristic features.

Physical characteristics of food grains are important in association with the design of a specific machine or analysis of the behaviour of the product in terms of handling and storage, and in the development of new consumer products. Fifteen genotypes of maize grain field (flint), sweet, and popcorn populations were evaluated for the physical characteristics of bulk density, true density, porosity, 100-grain weight, hardness and terminal velocity in the present study. An attempt has been made to relate these properties to the known features of different types of corn, heir specific usage and possible implications.

The average bulk density was measured by gently filling a 1000 cc container with the grain and then weighing it. The average true density was determined using the toluene displacement method (Mohsenin, Gordon & Breach Science Publishers, 1970). Porosity of husk was computed from the values of true density and bulk density using Eq. 1.

$$\varepsilon = [1-(\rho_b/\rho_t)]x100 \qquad ...(1)$$

where, ε = porosity, per cent

ρ_b = bulk density, kg/m^3

ρ_t = true density, kg/m^3

The hardness was measured as the first peak force recorded while a grain was compressed by a probe incorporated in a Texture Analyzer (Model TA+Di®, Stable Micro Systems, UK). The test speed of the probe was set at 0.5 mm/s and compression of grain was programmed for up to 60%. Five grains were compressed and the maximum value of first peak force recorded from force-time diagram among 5 grains was taken as the hardness of the grain. A typical force-time diagram is given in Figure 1. Terminal velocity was measured as the minimum air velocity at which the grains remained suspended in air.

Physical characteristics determined for 15 genotypes of maize at an average moisture content of 9.78% (wb) are presented in Table 1. Bulk density, which is an important parameter for determining the volume of storage containers, was found to be in the lowest range for sweet corn genotypes, except for the genotype in plot no. 963. Field corn genotypes had an intermediate density, and the popcorn genotypes had the maximum density. A majority of the sweet corn genotypes had the lowest true density, while the popcorn genotypes had the highest. Porosity values, which provide a measure of water requirement during any hydrothermal treatment, of sweet corn genotypes were the highest among the 3-grain types, except for the genotypes in plot no. 963. 100-grain weights of field corn genotypes were the highest followed by popcorn and sweet corn genotypes.

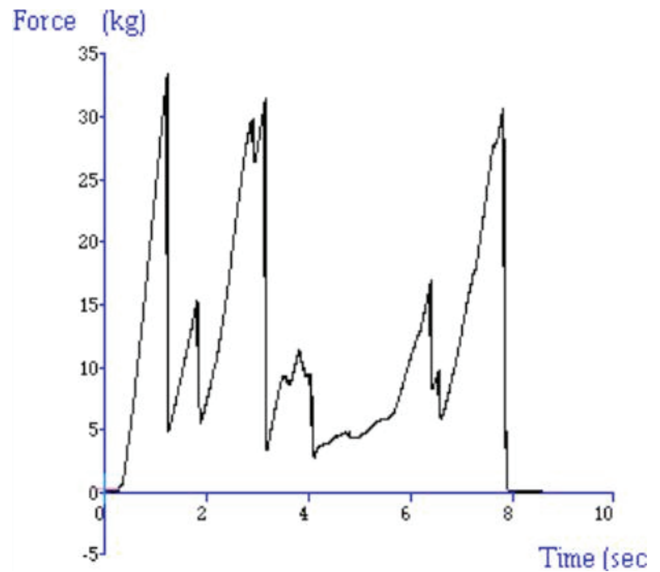

Figure 1. Typical force-time diagram for determination of hardness of maize kernels.

Table 1. Physical characteristics* of selected maize genotypes.

Genotype plot No. (*Kharif*-05)	Grain type	Bulk density (kg/m^3)	True density (kg/m ^{3})	Porosity %	100-grain weight (g)	Hardness (kg)	Terminal velocity m/s
957	Sweet corn	653.4	1276.3	48.80	15.14	37.83	12.0
958		666.6	1316.9	49.38	15.93	47.10	12.5
959		549.6	1112.9	50.61	16.30	41.65	11.0
960		525.0	1070.4	50.95	14.26	31.95	9.8
961		563.4	1127.7	50.04	13.41	32.65	10.0
962		610.2	1113.8	45.21	12.25	32.83	9.9
963		743.8	1226.7	39.36	23.30	32.32	13.5
964	Field corn (flint)	718.8	1293.9	44.44	25.87	37.77	14.5
965		736.2	1217.9	39.55	20.58	32.70	13.5
966		762.2	1308.0	41.72	23.89	63.33	14.5
967	Popcorn	797.2	1287.0	38.05	13.54	40.73	13.5
968		772.6	1303.2	40.71	16.12	44.71	13.7
969		711.0	1288.2	44.80	17.40	43.13	13.4
970		744.6	1270.9	41.41	13.84	40.90	13.7
971		742.8	1352.8	45.09	16.85	34.75	14.2

*Physical characteristics were determined at an average moisture content of 9.78% (wet basis).

Grain hardness plays an important role in product formulation and development of machines such as grinders, de-germers, etc. In general, hardness of popcorn and field corn genotypes was found to be greater than that of sweet corn, except for the genotypes in plots number 958 and 959. Characteristically, the genotype in plot no. 966 (field corn) was found to be the hardest, whereas the genotype in plot no. 960 (sweet corn) was the least hard.

Terminal velocity of grains is considered important for its separation with other grains. Sweet corn genotypes, except for the genotype in plot no. 963, had the least terminal velocity compared to popcorn and field corn genotypes.

Perusal of the results indicates some distinction of the sweet corn genotypes (lower hardness, test weight, bulk density and terminal velocity and higher porosity) from the other two types of corn. This may be related to and reflected by lower accumulation of starch in the kernels of these genotypes due to specific en-

dosperm mutations (Coe and Polacco, MNL 68: 157-208, 1994). This property, if confirmed by further studies, may be useful as a selection criteria for maize breeders, especially in efforts to convert field corns into sweet corns. It is relatively difficult to separate flints and popcorns on the basis of the physical characteristics elaborated above. This may be attributed to the presence of both hard and soft starches in popcorns, determining the popping property. Thus, there may not be a simple relationship between the physical characteristics of the kernels and popping features of popcorn.

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_Bg_ transposon: a possibility of regulation of transcription through formation of Z-DNA and Z-DNA binding properties of its encoded proteins

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Probable structure and properties of _Bg_-encoded proteins. Previously, on the basis of *Bg* transposon sequence, the primary structure of its putative encoded proteins (designated as PPBg1, PPBg2 and PPBg3; MNL 79) was proposed. The sequence of one of them, PPBg3, consists in fact of sequences of the two other proteins (PPBg1 at the N- end and PPBg2 at the C-end) divided by an insertion of 38 amino acid residues (MNL 79). Further analysis of this protein's sequence indicates that besides domains described earlier (DNA binding, catalytic activity, oligomerization; see MNL 79) it contains 2 regions similar to nuclear transport signals. The first one includes residues R13-R15 and H28-K32 (highlighted in bold in the sequence ***RRR***SNATVTDEQDDC***HRKGK***; see Fig. 1c in MNL 79) and resembles a nuclear localization signal (NLS) motif of the basic bipartite type (Yoneda et al., Cell Struct. Funct. 24:425-433, 1999; Xiao et al., J. Biol. Chem. 276:39404–39410, 2001). The structure of the second region (LxxxLxxLxL, residues L396-L405 of the sequence LVVALQFLVL; see Fig. 1c in MNL 79) is similar to the nuclear export signal (NES) of MAP (mitogen-activated protein kinase kinase) (Yoneda et al., 1999; Xiao, 2001; Perander et al., J. Biol. Chem. 276: 13015–13024, 2001).

Indicated NLS and NES signals are encoded by the two largest ORFs of the *Bg* transposon, located near its 5' and 3' ends. (For PPBg3 these ORFs constitute the first and the last exons and encode 84% of its sequence.) It is necessary to mention that nuclear transport signals are characteristic for large (e.g., PPBg3-like, the molecular mass of which is 69.4 kDa) proteins (see for example Yoneda et al., 1999). All this, even in the absence of experimental data on *Bg*-encoded proteins, strongly suggests that at least one of the products of this transposon should be a large protein, and that the two longest ORFs of *Bg* take part in encoding this protein sequence.

Similarity of a region in the N-end of _Bg_-encoded proteins to the Zα domain of Z-DNA binding proteins. Quite unexpected was the resemblance of a region in the N-end of probable *Bg*-encoded products PPBg3 and PPBg1 to the Zα domain of DNA binding proteins (Fig. 1). This region is encoded by a part of the longest 5' end ORF starting from the 813 position of *Bg* sequence (numbering of *Bg* element bases is given according to its GenBank accession number, X56877.1). In Figure 1 the comparison between PPBg3 and double-stranded RNA adenosine deaminase (ADAR1, showing the highest affinity of Z-DNA in comparison with other Z-DNA binding proteins used; see Kim et al., PNAS 101:1514-1518, 2004) is highlighted. It is necessary to mention that the part of the Zα domain used contains 10 out of 17 amino acids which are important for the protein fold and for Z-DNA recognition (see Kim et al., PNAS 100:6974–6979, 2003).

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PPBg3        71   VNKKSLFMVLYSCIFKILWSYTAG   94
hZα ADAR1   167   TPKKEINRVLYSLAKKGKLQKEAG  190

mZα DLM1     40   VPKKTLNQVLYRLKKEDRVSSPEP   62
Yaba         41   INKKKINQQLYKLQKEDTVKMVPS   64
Vaccinia     38   MEKREVNKALYDLQRSAMVYSSDD   61
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Figure 1. Similarity of the region V71-G94 of PPBg3 to the Zα family of Z-DNA-binding domains. Sequences used according to GenBank accession numbers (designated on the Figure 1 according to Kim et al., 2003) are as follows: AAB06697.1, the double-stranded RNA adenosine deaminase (ADAR1, $hZ\alpha_{ADAR1}$, (*Homo sapiens*)); NP_067369, Z-DNA-binding protein 1, tumor stroma and activated macrophage protein DLM-1 ($mZ\alpha_{DLM1}$, *Mus musculus*); NP_073419, the 34L protein (Yaba, Yaba-like disease virus); AAA02759, the E3L protein, (Vaccinia, vaccinia virus). Comparison between PPBg3 and ADAR1 is highlighted. Identical residues are shown on a black background, similar ones are on a gray background.

In connection with the finding of the Z-DNA binding motif in *Bg*-encoded products and the known ability of transposons for autoregulation (see for example Raizada et al., Mol. Genet. Genomics 265:82–94, 2001; Kunze and Weil, Pp. 565-610 in "Mobile DNA II", Craig et al. (eds.), ASM Press, Washington, 2002.) it is tempting to find out whether regions of the *Bg* transposon's sequence are able to form Z-DNA.

Several regions in the 5' end of the _Bg_ transposon sequence may potentially form Z-DNA. Analysis for the presence of Z-DNA forming regions in the *Bg* sequence was carried out using the ZHunt program (Ho et al., EMBO J. 10:2737-44, 1986; Ho, PNAS 91:9549-9553, 1994; Champ et al., Nucl. Acids Res. 32:6501-6510, 2004). (Access to this program was kindly offered by Prof. P. S. Ho from Oregon State University.) This analysis revealed two regions with Z-DNA forming potential: 1) positions 120-140 (ACCAGACGCGCGCACGAGAGC, Z-score $2.2 \cdot 10^4$); 2) positions 402-414 (CACGGACGCGCAG, Z-score $9.3 \cdot 10^2$) (Fig. 2, see also Table 1). For convenience, they will be further referred to as BgZDR120 and BgZDR402, respectively (for Z-DNA forming regions, ZDR; Champ et al., 2004).

These regions are present upstream of the translation initiation start site for PPBg3 (and PPBg1, position 813 of the *Bg* sequence) in the 5' end of the *Bg* element characterized by high G and C content (lacking the TATA promoter). This G/C rich region resembles the similar one of the *Ac* transposon (Kunze et al., EMBO J. 6:1555-1563, 1987) and some mammal housekeeping genes (see Hartings et al., Mol. Gen. Genet. 227:91-96, 1991; Maydica 36:355-359, 1991).

The possibility of the presence of other regions with Z-DNA forming potential in the *Bg* sequence, besides the ones found by the ZHunt program, cannot be excluded. Thus, taking into account the pattern of distribution of purine and pyrimidine bases in Z-DNA forming sequences (see for example McLean et al., PNAS 83:5884-5888, 1986; Schroth et al., J. Biol. Chem. 267:11846-

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  1 CAGGGAAAAC TTTATCGCCG ATAACACCTC CGATAAACCC GATTTTCCTG TTTATCGCTG
 61 GGCTCCGATT ATTTTACATA TCGGCCAAAA ATTTCGGCCC ATTTTGAATT TGGGCCCAAA
121 CCAGACGCGC GCACGAGAGC TAGGTTTTCT ACTGGCATTT GCTTGCAGCC GCCCGAGTCT
181 CCCTCCGTCC GAATCACATT CAATCTCTCT CCACTCCCCA GTTCCCACCT GAGTACCCGA
241 CGCCGCACTG CCGCAGCCGC CGCCGTCCAG CTCGGCAGCT CGTGCCCTCG CCCCTCCGTC
301 AAGAAGCGAG CTCGACCCCG GCCCCCCGGC GCACCTGCTC CTCGCTGCGG CTACGCTCAT
361 CGCGGAGTGG CGGCCTGGCG GACGACTCCG CGGGCCGGCG CCACGGACGC GCAGCGCCAG
421 GGCTACTTCG AGGCCGAGCT CGTCCACGGC GAGTACAAGG TCACCCGTCA CCGTCAGCCA
481 CCCGGCGGCT CAACAGCTCC ATCTCCCAGA GCGTCAAGGT GGAGCTGGGC TCAGGGAGTG
541 ACCAACTGAC CGCTACTTCA TTGACATGCA GGTCTGTCTG GGGTGTGCCG CGAGGAGTGG
601 CGAGACCACG AGAGCAGCAG GAGGTTGACG GGGAAGGGGA CAACCAGAAC CAGCACGTGC
661 GACGCCAGGG TAGGTGTGGC TGCTGTCTCC ATGCCGCACA GCTCCACTGC AGCTGCTGTC
721 TCATTTTTTT ATAACTAGAA ATATTAAAAA TAGATGCTTG CTCTCTGGTT AATTTACAAT
781 TACTTGAGAT GATTGTAGAA AGTTAGAAAC ACATGGCATT TGAGGTTGAG GAAGACGATG
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Figure 2. A part of the 5' end of the *Bg* transposon sequence (residues 1-840).The probable Z-DNA forming regions (BgZDR120 and BgZDR402 counting from the 5' end, respectively) revealed by the ZHunt program (Ho et al., 1986; Ho, 1994; Champ et al., 2004) are dashed. Sequences similar to the Inr and DPE elements are in bold italic. The translation start site for PPBg3 (and for PPBg1) is in bold.

11855, 1992; Herbert and Rich, J. Biol. Chem. 271:11595-11598, 1996; Champ et al., 2004) several regions in the *Bg* sequence (e.g., sequences found near BgZDR402 region, positions 388-423, CGCGGGCCGGCGCCACGGACGCGCAGCGCCAGGGC; downstream of this region in positions 579-592, TGGGGTGTGCCGCG and positions 674-686, GTGTGGCTGCTGT) could be distinguished as having the propensities for forming Z-DNA. It is possible that these regions may form Z-DNA in the presence of certain specific cellular factors.

It is known that Z-DNA can be generated by transcription and that distribution of Z-DNA is nonrandom, with its preference for localization near transcription initiation sites (Wittig et al., PNAS 88:2259-2263, 1991; Schroth et al., 1992; Champ et al., 2004).

Transcription start site for *Bg* transcripts. Works on transcription initiation sites for *Bg*-encoded products are unknown to the author. However, taking into consideration the conserved motifs of initiator (Inr) and downstream promoter elements (DPE), the sequences which commonly are present in TATA-less promoters (see for example Zhang and Dietrich, Nucl. Acids Res. 33:2838-2851, 2005; Kadonaga, Exp. Mol. Med. 34:259-264, 2002; Nakamura et al., Plant J. 29:1-10, 2002), at least two motifs similar to the Inr elements located in good agreement with the DPE resembling motifs could be distinguished in the 5' *Bg* transposon end (Fig. 2).

One of these motifs is situated near the BgZDR120 region, the other one is near the BgZDR402 region, confirming the above-mentioned predisposition of Z-DNA for localization near transcription initiation sites. In the first Inr-like sequence (positions 116-122, CCA$_{+1}$AACC), the last three base pairs enter in the BgZDR120. The G-139 of the sequence similar to the DPE element (positions 139, 143-147, G$_{+22}$, GGTTT) is also a part of the BgZDR120 and is situated 2 base pairs (bp) closer to A-118 of the first Inr-like sequence in comparison to the consensus DPE motif of *Drosophila* (G$_{+24}$, (G/A)$_{+28}$G(A/T)(C/T)(G/A/C); Kadonaga, 2002). The second Inr-like sequence (positions 423-429, CTA$_{+1}$CTTC) is located 9 bp downstream of the BgZDR402 region and is followed by the DPE-like motif of ***G***$_{+24}$GCG***AGTAC*** (positions 448-456).

Possible mechanisms of regulation of *Bg* transcription through the interaction between Z-DNA-binding domains of its encoded proteins and its Z-DNA forming regions. Formation of Z-DNA can activate transcription and act as the *cis*-element in genic regulation (Liu et al., Cell 106:309-318, 2001; Sheridan et al., Mol. Microbiol. 40:684-690, 2001; Oh et al., PNAS 99:16666-16671, 2002). Z-DNA binding proteins can stabilize Z-DNA and act as potent effectors of gene expression (Oh et al., PNAS 99: 16666-16671, 2002). On the other hand, the inhibitory action of Z-DNA on promoters is also known (Sheridan et al., 2001; Rothenburg et al., PNAS 98:8985-8990, 2001). Therefore, the determination of the real character of interactions between Z-DNA and Z-DNA-binding domains of *Bg*-encoded proteins and the effects of such interactions on the promoter activity need, of course, experimental studies.

However, taking into account that the activity of the *Bg* transposon can be assessed by the rate of excision of the nonautonomous *rbg* element from its mutable *o2-m(r)* alleles (leading to reversion of these alleles to the normal one), some assumptions could be made proceeding from features of the reversion of *o2-m(r)* alleles in the presence of *Bg* elements. Reversion of such alleles is characterized by the specificity of their interaction with different *Bg* transposons and can strongly depend on the dosage of these autonomous elements (Maydica 44:195-203, 1999; Maydica 48:275-281, 2003; Genetika (Moscow) 39:769-774, 2003). Two features of observed dosage effects can be underlined: 1) a significant, outstripping increase of reversion frequency of the *o2-m(r)* alleles when the dosage of *Bg* transposons increases from 1 to 3 or from 1 to 2; 2) an insignificant change in reversion frequency of the *o2-m(r)* alleles when the dosage of *Bg* transposons increases from 2 to 3 (see for example the behavior of the *o2-hf* allele in the presence of *Bg-hf*; Maydica 44:195-203, 1999; Maydica 48:275-281, 2003; Genetika (Moscow) 39:769-774, 2003).

Previously, several suppositions were made about the properties of *Bg*-encoded product(s) that explain the above-mentioned features of *Bg* dosage effects on the posttranscriptional level of its encoded products (Maydica 48:275-281, 2003; Genetika (Moscow) 39:769-774, 2003). However, finding the Z-DNA binding domains in *Bg*-encoded proteins and the ability of certain regions of *Bg* sequences to form Z-DNA may indicate the existence of mechanisms of regulation of *Bg* dosage effects on another, transcriptional level.

Thus, assuming that the formation of Z-DNA near transcription start sites enhances the efficiency of transcription by maintaining open confirmation of chromatin in this region (see Liu et al., Cell 106:309-318, 2001) and that there is a positive dependence between frequency of *rbg* excision and concentration of *Bg*-encoded proteins, a positive autoregulation mechanism for transcription of the genes encoding *Bg* products can be proposed. By this

mechanism, transcription efficiency is enhanced by the increased stability of Z-DNA due to an enhancement in the stabilizing action on Z-DNA of the Z-DNA binding *Bg*-encoded proteins when the *Bg* dose increases from 1 to 2 or from 1 to 3.

Several explanations are possible concerning the role of Z-DNA located downstream of transcription start sites in the *Bg* sequence. The presence of these Z-DNA regions may increase fidelity of RNA splicing by a mechanism proposed by Wittig et al. (1992). Another explanation can be prompted by the aforementioned feature of an insignificant change in reversion frequency of the *o2-m(r)* alleles when *Bg* dosage increases from 2 to 3. For example, binding of *Bg*-encoded proteins at their enhancing concentration (when the dose of *Bg* elements increases from 2 to 3) to the indicated Z-DNA regions (especially if these regions show lower affinity to *Bg*-encoded proteins and are bound by such proteins at their high concentration) may hinder the movement of the next RNA polymerase molecule on these regions, thus lowering the efficiency of transcription. That is, the stabilizing action of *Bg*-encoded proteins on Z-DNA situated downstream of transcriptional start sites would affect gene transcription in a negative autoregulation mode.

In any case, the presence of Z-DNA binding domains in *Bg*-encoded products, and the ability of certain regions of the *Bg* transposon to form Z-DNA, may indicate the existence of mechanisms of *Bg* activity autoregulation through the interaction of Z-DNA forming regions of this transposon with its encoded proteins.

Z-DNA forming regions in other maize transposons sequences. Using the ZHunt program a search for Z-DNA forming regions in sequences of other maize transposons (*Ac*, *PIF*, *En*, *MuDR*) was carried out. Such regions were found in sequences of all transposons analyzed except *PIF* (GenBank accession number AF412282.1) (Table 1).

Table 1. Probable Z-DNA forming regions in sequences of different maize transposons revealed by the ZHunt program (Ho et al., 1986; Ho, 1994; Champ et al., 2004).

Transposon	Starting position	Length, bp	Z-score	Sequence
Bg	120	21	$2.2 \cdot 10^4$	ACCAGACGCGCGCACGAGAGC
	402	13	$9.3 \cdot 10^2$	CACGGACGCGCAG
Ac	381	16	$3.3 \cdot 10^3$	CCACGCGCCCACGCCG
	1261	28	$1.4 \cdot 10^5$	ATGTACGTGCACGTGCGCGTGGGCATGG
En	397	15	$1.8 \cdot 10^3$	GAGCGCGCACCTCCA
	5892	13	$4.7 \cdot 10^3$	TTCGCGTGTGCGA
	8035	17	$2.4 \cdot 10^3$	TGATGTGCGCGCAGTAA
MuDR	169	19	$1.8 \cdot 10^4$	TTCGCCCGCGCACACGCCG
	4756	20	$1.8 \cdot 10^4$	CGGCGTGTGCGCGGGCGAAC

Sequences used (according to the GenBank accession numbers) are as follows: X56877.1 (*Bg*); X05424.1 (*Ac*); M25427.1 (*En*); M76978.1 (*MuDR*). The Z-Score cutoff (minimum) is equal to 700.

Interesting results are observed for potential Z-DNA forming regions of the *MuDR* transposon. A characteristic feature of this transposon is the convergent transcription of its two major transcripts, *mudrA* and *mudrB*, initiated in terminal inverted repeats from opposite strands (Hershberger et al., Genetics 140:1087-1098, 1995). Transcription start sites of these transcripts (Hershberger et al., 1995) are located near revealed Z-DNA forming regions: the beginning of the first Z-DNA forming region (starting from position 169 of the *MuDR* element sequence, see table 1) coincides with the starting bp nucleotides of the first start site of the *mudrA* transcript; the second Z-DNA forming region (positions 4756-4775 of the *MuDR* element sequence, see Table 1) is located 5 bases downstream of the transcription start site of *mudrB* (position 4780; Hershberger et al., 1995). These results confirm one more time the predisposition of Z-DNA for transcription start sites and indicate the involvement of Z-DNA in the regulation of the transcription of the *MuDR* transposon genes.

Corrigendum. In the MNL 79 note on *Bg*-encoded proteins a misprint was made in the legend of Figure 1: the correct numbers for the first exon of PPBg3 are 813-1546.

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A seed-by-seed strategy to study the paramutation at *r1* locus
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The paramutation at the *r1* locus was largely studied by classical and molecular approaches, and several aspects of its behavior and origin were elucidated. Classical experiments provided information about the locus structure, genetic distance and phenotypical instability, and the molecular genetics revealed the sequence and the elements that comprise the locus, including transposable elements, genes and methylation. Many advances have been made towards the understanding of the control of the paramutation at the *r1* locus, however many questions remain unanswered. One question that has interested us is related to the instability of the phenotypes after the paramutagenic allele altered the paramutable one in the heterozygous state. Seven classes of kernel pigmentation are known, varying from colorful with a maximum deposition of anthocyanin to colorless without pigmentation, in the F2 progeny. The literature is categorical in descriptions about reversions to the original state of the allele after several cycles of self-pollination, it being well established that the paramutation is an unstable event. The knowledge about stable paramutant alleles is incipient and the selection of a stable phenotype of each class of pigmentation could represent material important for molecular investigation. In this work, the main aim is the development of a strategy that permits us to understand the instability of the alleles and the selection of possible stable alleles to produce inbred lines that could be used in the molecular investigation.

We had previously described the introgression of a paramutable *r* allele in traditional varieties of maize from Brazil (Gardingo and Mondin, MNL 77:60-61, 2003). Inbred lines have been derived from the varieties that express the paramutation. The phenotypical classes have been scored from 1 (colorless) to 7 (colorful) and every classes was observed in the S1. To obtain the S2, a bulk of the classes 3, 4 and 5 was selected. In the S3 generation each class was followed seed-by-seed and evaluated as to the seven seed color pigmentation classes. Here, we present some results in one inbred line derived from the Carioca variety (Ca).

The S2 generation was scored considering the ear as a whole. From all patterns of segregation expected, only six were observed (Table 1). In one case, a pattern did not present colorless seeds, and the seeds in the ears segregated from class 2 to class 6. A high number of colorless ears were recovered, which could be a

Table 1. S2 segregation pattern, derived from a bulk of seed of pigmentation color classes 3, 4 and 5.

	Segregation Mode*					
Inbred Line	Colorless	Colorless/2-4	Colorless/2-5	Colorless/6	2-6	All Classes (1 to 6)
Ca	81	47	71	13	5	19

* Number of ears scored.

result of the homozygous recessives. A complete imprint was not discarded, but new experiments should be conducted to consider this hypothesis. No colorful seed was recovered in any ear. Class 6 was scored in a low frequency, and the seeds were self-fertilized to observe the pattern of segregation in the S3 generation. Even in the ears segregating to different classes, the highly pigmented seeds were recovered in a low frequency. To exemplify this, the ears segregating colorless/2-5 were scored seed-by-seed. Table 2 presents the absolute numbers, and Figure 1 shows the average of each class from the ears. It is clear that the frequency of the class 5 was significantly lower than the other classes. We have considered this case as a pattern of segregation, since several ears have shown similar frequencies. We expected a higher frequency of reverting seeds, expressing color classes 6 and 7. We have postulated that the effect of the paramutable allele is very strong, and several generations of self-fertilization were needed to recover the colorful class.

Table 2. Total of seeds scored on the colorless/2-5* ears.

	Seed Color Classes			
Inbred line	Colorless	2-4	5	Total
Ca	7221	7230	674	15125

*Presented in the Table 1

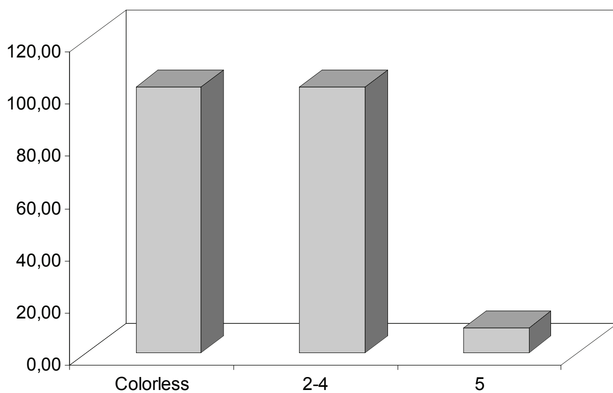

Figure 1. Average frequency of seeds from different classes of pigmentation scored in colorless/2-5 ears.

In the S3, colorful seeds were not recovered, however classes 5 and 6 were more frequent. The number of non-segregating ears was higher (Table 3). Seed class 1 resulted only in colorless ears. This case has been interpreted to be recessive homozygous, but this class has been analyzed carefully to identify possible reversions to color classes. Every seed class scored segregated colorless. We were expecting a higher frequency of classes 5, 6 and 7, but as they were not observed, the postulate described above seems to be true.

Analyzing seed-by-seed the non-segregating S3 ears derived from class 3 seed, a lower frequency of seeds in class 5 and a higher frequency in class 4 was observed, while ears derived from class 5 seed presented a lower frequency of seed in the less pigmented classes (Table 4). Class 4 ears showed segregation for classes 2 to 6. Some ears showed a unique class of seed color, mainly when derived from class 5, and these ears have been selected for evaluation. The most important observation was the absence of colorless seeds. The classes observed in a non-segregating ear should be analyzed seed-by-seed, trying to minimize the segregation to different color classes. These results indicate that the selection of some seed color classes in non-segregating ears, followed by seed-by-seed analysis in the next generation, could be a good strategy to stabilize the paramutation. Some crosses between plants of the same class have generated seeds of the same class (data not shown), for example, crosses of class 4 produced ears fully class 4.

Table 3. S3 segregation pattern derived from selected S2 seed color classes.

Seed Color Classes	Ears Scored	Segregating to colorless	Non-segregating	Colorless
1	40	0	0	40
2	16	14	1	1
3	50	39	7	4
4	30	24	5	1
5	11	7	4	0
6	4	4	0	0

Table 4. Frequency of seed color classes on S3 non-segregating ears.

S2 seed color	Seed Color Classes				
classes	2	3	4	5	6
3	30	322	264	23	0
4	15	121	497	305	10
5	0	25	75	305	88

We believe that as a stabilized paramutation is obtained, some important aspects of the *r1* paramutation will be explained, such as the role of transposable elements in paramutation events. Moreover, the stabilization of a phenotype class could be a result of a chromatin conformation that is transmitted generation to generation without alteration, or a suppression of the recombination among the repeats of *r1*. All these interesting questions could be investigated utilizing these stable lines with molecular approaches.

PRESIDENTE PRUDENTE, BRAZIL
University of Western Sao Paulo

Changes in chromosomes in highly embryogenic cultured cells and in germinating stored seeds of maize

--Scandolieri, RF; Koyanagui, AP; Takahashi, FT; Fluminhan, A

In higher plants, an increased frequency of genetic and chromosomal changes is usually observed during the germination and development of plants derived from aged seeds and regenerated from in vitro cultured cells. Much evidence supports a close relationship between the age of stored seeds or cell cultures and the loss of vigour and germinability of seeds, and the regeneration ability of cultures and number of chromosome aberrations that are observed at the first mitoses of root meristems in surviving plants.

In vitro culture of plant cells has led to several useful approaches for biotechnology in the agricultural sciences, such as: the selection and clonal propagation of promising genotypes and

the production of transgenic cultivars. However, it is necessary that the cell cultures maintain their ability to regenerate fertile plants without showing any genetic variation, even after long periods of in vitro culture. Cytogenetic analysis of regenerated plants and their progenies and in germinating aged seeds has allowed the identification of several chromosome number variations and changes in chromosome structure.

We have investigated the occurrence, nature and frequency of chromosome abnormalities in mitotic anaphases in both systems: highly friable and embryogenic (type II) callus cultures and aged seeds of a maize synthetic cultivar obtained from a breeding program developed at our university. In vitro performance of the Unoeste 101 cultivar was evaluated following standard protocols for callus induction from immature embryo, and the embryogenic callus cultures have been maintained for more than 32 months, and continuously evaluated for their ability to regenerate complete plants. Samples of root meristems collected from regenerated plants and from germinating aged seeds (stored in culture rooms at 25° C, for 3 years, with moisture content brought to approximately 8% by drying over regularly regenerated silica gel) were taken for cytogenetic analysis, and the squash preparations were stained by Feulgen's and C-banding methods.

The results demonstrate the occurrence of extensive mitotic abnormalities in both systems (cultured cells and aged seeds), mainly as a result of the formation of bridges that may lead to chromosome breakages (Fig. 1). Previously, we have demonstrated that the primary chromosome breakages occur preferentially within knobs or at junctions between the euchromatin and a heterochromatic knob (Fluminhan et al., Ann. Bot. 78:73-81, 1996; Fluminhan and Kameya, Theor. Appl. Genet. 92:982-990, 1996; Fluminhan and Kameya, Genome 40:91-98, 1997).

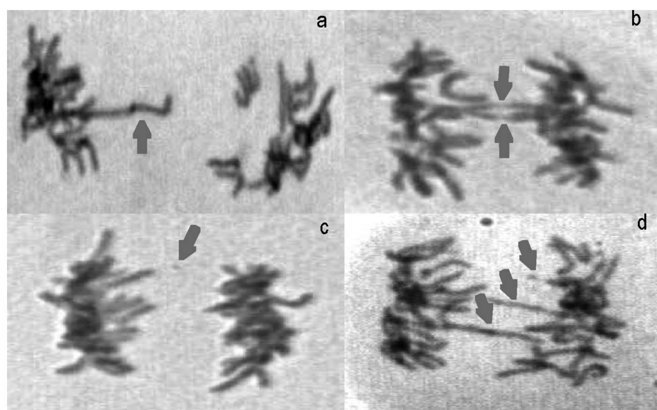

Figure 1. Photomicrographs of mitotic anaphases observed in cultured cells (**a** and **b**), and at first mitoses of germinating aged seeds (**c** and **d**) of the maize cultivar Unoeste 101. **1a**. Typical anaphase showing the primary event, characterized by a delay in segregation of sister chromatids, resulting in a lagging chromosome (arrow). **1b**. Typical anaphase showing double bridges. **1c**. Chromosome breakage giving rise to a fragment (arrow). **1d**. Typical anaphase with multiple bridges, with and without heterochromatic knob, apparently resulting from successive breakage-fusion-bridge cycles. Magnification: x100 objective; x10 ocular; x1,2 additional lens.

These findings are consistent with our previous observation of an increased presence of methylated cytosine at knob heterochromatin (Fluminhan et al., MNL 71:75-77, 1997) and with the proposition that changes in the degree and pattern of DNA methylation could be an underlying cause of chromosomal abnormalities observed in cultured cells and regenerated plants (Phillips et al., Proc. Intl. Cong. Plant Tissue Cell Cult. 7:131-141, 1990).

Our observations indicate that all the phenomena described above resemble each other in both systems analysed, and represent an important step aiming the proposition of mechanisms related to their occurrence. One interesting issue to be analysed is that both systems could be under the influence of common or related mechanisms of cellular senescence, leading to the occurrence of similar abnormalities at mitosis.

RALEIGH, NORTH CAROLINA
North Carolina State University

Effects of plant growth regulator 2,4-D, KT and BA on callus induction and plant regeneration from mature embryos of maize

--Wu, M-S; Wang, X-F

Genetic engineering of maize is dependent on the development of efficient and reliable callus induction and a plant regeneration system. Mature embryos are a potentially useful alternative to immature embryos because they can be stored in the form of dried seeds and are available at all times. In the present work, we investigated the effect of auxin and cytokinin on maize callus induction and plant regeneration from mature embryo of maize.

Inbred lines A188, B73 and their hybrid (A188 X B73) were used as the source for mature embryo culture. The seeds were surface-sterilized for 1 min in 75% ethanol and rinsed three times with sterilized, deionized water. The seeds were then surface–sterilized for 10 min in 10% (v/v) commercial bleach containing two drops of Tween 20 followed by six rinses with sterilized, deionized water. Mature embryos were excised from the seed and placed onto the surface of callus induction medium (IM). The IM was MS medium supplemented with 2,4-D at different concentrations (1, 2, 4, 6, 8, 10 and 12 mg/L), KT (0 to 0.5 mg/L), 3% sucrose, and 0.8% agar. After 8 days, root and bud were removed from mature embryo. Cultures were transferred at two-week intervals to fresh IM medium. All the cultures were incubated at 26ºC in darkness. After calli were induced, all calli were transferred to regeneration medium to induce plantlets and placed in a growth chamber at 26ºC. Regeneration medium contained MS inorganic salts at half strength supplemented with the same organic nutrients as the callus induction media and the cytokinin benzylaminopurine (BA) at different concentrations (0, 0.2, 0.4, 0.6, 0.8, 1 and 2 mg/L). Regenerating calli were cultured in a growth chamber at 26ºC with light. Plantlets with developed roots were transferred to a sand-soil mixture in plastic pots and placed in the growth chamber for two weeks at 26ºC. After two weeks, plants were transplanted and grew in the greenhouse till setting seed.

Callus induction was strongly influenced by the type and dose of auxin. Two main types of calli were observed in the cultures (non-embryogenic and embryogenic callus). Fresh weight of calli increased when the concentration of 2,4-D was raised from 1 to 4 mg/L, while the rate of embryogenic calli decreased when the concentration of 2,4-D was changed from 6 to 12 mg/L. The medium containing KT was effective in promoting a greater frequency of embryogenic callus. The combination of 2,4-D (4 mg/L)+ KT (0.5 mg/L) was the most effective for producing embryogenic callus. Genotype was closely related to callus production from the mature

embryo. More calli were induced from the mature embryos of inbred lines A188 and B73 than those of hybrid line A188 X B73. For plant regeneration, BA had a significant effect on the regeneration of embryogenic callus; a high concentration of BA (1mg/L and up) caused a decrease in the rate of regenerated plantlets. Rate of plantlet regeneration was the highest in the medium containing 0.6 mg/L of BA. Under the most optimal conditions, the frequency of plant regeneration could be as high as 25.7%, 33.1% and 21.6% for inbred lines A188 and B73, and their hybrid line (A188 X B73), respectively.

In conclusion, it was shown that auxin and cytokinin concentration had significant effects on callus induction and plant regeneration from mature embryos in maize. The greatest callus growth occurred when the medium included 2,4-D, at a 4mg/L concentration, in combination with KT (0.5 mg/L). The addition of BA (0.6 mg/L) in regeneration medium was the most effective in promoting plant regeneration of embryogenic callus in this study.

SAINT PAUL, MINNESOTA
University of Minnesota

How many maize genes are not in B73?

--Okagaki, RJ; Schmidt, C; Stec, AO; Rines, HW; Phillips, RL

We are finding evidence that the genomic sequences for numerous maize ESTs are not present in the B73 genome. These gene-like sequences can be found in other maize lines, but they appear to be missing in B73. In retrospect, it is not surprising to find deletion polymorphisms for maize genes. Polymorphisms for single base changes and simple sequence repeats are common, and deletions are merely another type of molecular lesion. Our results, based on a study of ESTs from A188, suggest there could be many gene-like sequences in the A188 inbred line that are partially or entirely deleted in B73.

Computational analysis identified gene-like sequences that were likely to be absent in B73; this was followed by a molecular analysis to determine if the sequences were indeed absent in B73 and other maize lines. Approximately 16,000 EST sequences that had been annotated as having come from A188 were downloaded from GenBank. EST sequences longer than 300 nucleotides were then searched against the maize B73 genomic sequences in the TIGR Maize Database, AZM Release 5.0. Approximately 500 EST sequences longer than 300 basepairs did not match a B73 genomic sequence with a probability value of e^{-10} or less, and thus may identify a maize gene that is not in the B73 genome. These sequences were matched with the EST contigs developed by TIGR, and the 521 sequences corresponded to 71 singletons and 156 contigs. BLAST searches were then repeated using the entire contig sequences to eliminate additional sequences. PCR primers were developed against the remaining 63 EST sequences, and PCR assays were used to detect the presence of these sequences in genomic DNA from A188, B73 and 12 other maize lines. We obtained results with 53 sets of primers. No products from B73 genomic DNA were detected with 17 primer sets, and 28 primer sets failed to amplify products from at least one maize line. Twenty-five primer sets amplified products from all 14 lines tested. Table 1 presents a sample of the data. We are in the process of confirming these results by Southern blot analysis. To date, Southern blot analysis has confirmed the absence of five sequences in B73 out of the 10 sequences tested. Extrapolation of these results to the approximately 50,000 maize genes suggests there could be many gene-like sequences in A188 that are missing in B73.

Table 1. Detection of A188 ESTs in maize lines.

EST sequence	A188	A632	B37	B73	C103	CML5	CML52	CML91	Mo17	Oh43	Tzi18	Co159	Tx303	W64A
CK700895	+	+	-	+	-	-	-	+	-	+	-	-	+	+
CB179401	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CB179394	+	-	+	+	+	-	-	+	+	+	-	+	+	+
DR906760	+	-	-	+	+	+	+	+	+	-	+	+	+	+
CN845215	+	+	+	+	+	+	+	+	+	+	+	+	+	?
CF349054	+	-	-	-	+	-	-	+	+	-	?	+	-	-
BM660009	+	-	-	-	-	-	-	-	-	+	+	-	+	+
CF920121	+	+	+	+	+	+	?	+	+	+	+	+	+	+
DN559697	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DN559490	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DN475032	+	+	-	-	+	-	-	+	-	+	-	-	+	-
DN586584	+	+	-	-	+	-	+	+	-	+	-	-	+	-
CD052452	-	-	-	-	+	-	-	-	-	+	-	+	-	-
CD052345	+	+	-	-	-	+	-	-	-	-	-	-	-	-
CF974781	+	-	-	-	+	+	-	+	+	+	+	+	+	-
CF273223	+	+	+	-	-	-	-	+	-	-	-	+	+	+
CN845203	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CV072139	+	+	+	-	+	+	?	+	+	+	+	+	+	+

These data are inadequate to estimate accurately the number of A188 gene-like sequences absent in B73. The BLAST analysis used to identify sequences that are present in B73 may underestimate the number of A188 sequences missing in B73. We used a cutoff of e^{-10} for identifying hits between A188 EST sequences and B73 GSS sequences. The BLAST threshold permitted hits between an A188 EST sequence and related, but not identical, sequences in B73. Many deletion polymorphisms occurring in duplicated genes could be missed. A study by Lai and co-workers (Genome Res. 14:1924, 2004) estimated that 50% of the genes duplicated in the maize tetraploid ancestor remain duplicated. Our search for A188 sequences missing in B73 may have ignored half of the maize genes. If the frequency of deletion polymorphisms was the same in single-copy and duplicated genes, then estimates of the number of A188 gene-like sequences missing in B73 should be doubled. The assumption that the frequency of deletion polymorphisms is similar in single copy versus duplicated sequences may not be valid; one study of human genes found that gene-deletion polymorphisms were 10-fold more common in segmentally duplicated regions of the human genome (Tuzun et al., Nature Genetics 73:727, 2005). Lastly, the polymorphisms observed here were unlikely to have been produced by the replication and transposition of sequences by *helitron* elements. *Helitron* elements do create polymorphisms similar to those observed here (Lai et al., PNAS 102:9068, 2005), however we are looking primarily at single copy sequences rather than the duplicated sequences associated with *helitron* transposition. In conclusion, it is likely that a full sequence of the B73 inbred line will necessarily miss a large number of the genes present in the species.

SARATOV, RUSSIA
Saratov State University

The possibility of producing tetraploid analogies from maize parthenogenetic lines

--Tyrnov, VS; Kolesova, AY; Smolkina, YV

We reported earlier about the production of maize lines predisposed to reduced parthenogenesis and autonomous endospermogenesis (MNL 71, 1997; MNL 75, 2001; and other articles in Russian). The frequency of parthenogenesis can reach 100%. The sign is nuclear and can be transferred to other lines by egg or pollen. On the basis of these lines, we carried out work on the production of apomictic maize forms. According to the literature, higher levels of ploidy than 2n (from 3n and higher) are characteristic for many apomictic species. Therefore, we have made experiments on the production of tetraploid analogies for previously obtained diploid parthenogenetic lines (AT-1 and AT-3). We have demonstrated before that after treating parthenogenetic diploids with colchicine, a great number of maternal-type diploid plants appear (MNL 77, 2003). The same results were obtained in the experiments in the next year. This indicates indirectly that the ability for parthenogenesis was not lost by the formation of diploid eggs.

In addition, we carried out research on the production of tetraploids forming spontaneously through the use of unreduced pollen. This approach is interesting, as it does not exclude the probability of production of lines with genes for non-reduction, which can be useful in the subsequent work of producing unreduced apomicts. As the maternal form, we used the ordinary tetraploid with yellow kernels. It was pollinated by pollen of line AT-1, characterized by purple color of plants and kernels. Such crosses were used for control in the event of occasional pollination by pollen from maternal tetraploids. Along with triploids, a small number of tetraploids were produced, which were self-pollinated. In self-pollinated progeny in three plants, 219, 189 and 124 embryo sacs were isolated by the ovule enzymatic maceration method, and were analyzed. In two embryo sacs of one plant we observed autonomous embryogenesis. In one of these embryo sacs the cellular embryo was found. In the second embryo sac, we observed simultaneous development of two embryos (6- and 8-cellular). In another plant one case of autonomous endospermogenesis (0.8%) was observed. Cases of atypical organization of embryo sacs that are characteristic for the initial diploid parthenogenetic line were also observed: synergid-like eggs with the nucleus in the basal position (2.1% and 4.1% in two plants), additional (7 and 8) cells in the egg apparatus, and additional (3, 4 and 5) polar nuclei. In all three plants there were very large 1-4 nuclear cells adjoining the antipodal apparatus (1.% - 4.0%).

Thus, the direct cytoembryological analysis has shown that in tetraploid analogies the ability for parthenogenesis, autonomous endospermogenesis and other characteristics of the embryo sac can manifest in the same way as in the initial diploid lines.

The possibility of the connection of additional cells with apospory and the reasons (heterozygosis, age of eggs and others) for relatively low frequencies of parthenogenesis and endospermogenesis are not clear for the present, and require additional research.

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In vivo and in vitro comparison of the heterotic effect in sweet corn

--Nedev, T; Krapchev, B

Plant tissue culture techniques have been used as a complementary tool in plant breeding improvement. Our aim was to study this technique as a procedure for early prediction of heterosis in sweet corn hybrid breeding programs. The genetic potential of sweet corn for initiation and maintenance of calli and regeneration of whole plants was investigated. A tissue culture experiment with mature embryos was used to study the possibility for prediction of heterosis at early stages of mature embryo growth. A half diallel set of crosses was made among ten sweet corn inbred lines, and 45 F1 hybrids were obtained. The kernels were divided into 2 groups for in vitro callus induction and for in vivo measurement of some agronomic traits. The kernels in the first group were evaluated for callus formation, and the growth rate of the calli obtained was measured. The potential for callus initiation was investigated on N_6 medium, supplemented with 4 mg/l dichlorophenyl acetic acid (2,4-D), vitamins according to Murashige and Skoog, 150 mg/l asparagine, 30 g/l sucrose and 7 g/l agar. Callus growth rate was estimated as an increase in the original fresh weight of the calli for a period of 30 days.

In general, sweet corn lines and F1 hybrids may be derived in 3 groups. The first group comprised kernels was capable of initiating callus; the second group consisted of kernels capable of initiating calli, which can grow at different rates; and the kernels in the third group were able to initiate callus with morphological characteristics totally different from the others. The potential of this third cross to initiate callus was investigated on immature embryos under conditions described by Nedev et al. (MNL 75). The cross was competent at initiatng morphogenic callus, and in vitro organogenesis was obtained. A study on the utility of this cross in in vitro programmes for improving maize breeding processes is in progress.

The ten sweet corn lines investigated and their respective 45 F1 hybrids were grown at the Experimental Farm of the Institute of Genetics in Sofia. Fifteen competitive plants were taken for recording data for the following characters: ear length (cm), ear circumference (cm), number of rows/plant, number of kernels/row, plant height (cm), tassel height (cm), ear height (cm), number of internodes, and length and width of the ear leaf (cm). From all 45 F1 hybrids investigated, 10 indicated a high heterotic effect regarding the majority of the characteristics tested. All hybrids surpassed the parental lines in the parameters investigated.

The preliminary data show that there is a positive correlation between the characteristics studied in vitro and in vivo.

STUTTGART, GERMANY
University of Hohenheim
FREISING-WEIHENSTEPHAN, GERMANY
State Research Center for Agriculture

Variation for female fertility among haploid maize lines

--Geiger, HH; Braun, MD; Gordillo, GA; Koch, S; Jesse, J; Krützfeldt, BAE

Progress in in vivo haploid induction by specific pollinator genotypes (inducers) has made it possible to routinely produce large numbers of maternal haploid (H) plants. Treating these plants with colcicine and selfing them leads to doubled haploid (DH) lines, which are highly efficient tools in genetic research and practical breeding (Eder and Chalyk, TAG 104:703-708, 2002; Röber et al., Maydica 50, 2005, in press).

Chalyk and Rotarenco (Plant Genetics 37:1382-1387, 2001) reported about a recurrent selection procedure based on H-plants as test units. H-plants were grown in an isolated plot surrounded by diploid plants of the parental breeding population. Seeds grown on H-plants fertilized with pollen from diploid population plants were used to establish the next selection cycle. This was possible in 10-30% of the H-plants. No information was given about the number of seeds obtained from these plants. Since haploids in higher plants generally are female and male sterile, the expected number should be close to zero. However, according to Chalyk (MNL 73:53-54, 1999) grain number may vary "between several and several dozen". In the present newsletter we are reporting about a multi-location field experiment in which we determined the seed set, thousand-grain weight (TGW) and grain yield of unselected H-lines.

The genetic material for our study was kindly provided by three collaborating maize breeding companies. It was derived from three elite dent single crosses adapted to the Central European climate. In the first step, about 80 DH lines were produced from each single cross by means of *in vivo* haploid induction using the proprietary inducer line RWS as pollinator (Röber et al., see above). In the second step, each DH line was converted to the haploid stage by again using the before-mentioned technique. Fifty-four to fifty-eight H/DH-line pairs per population had enough seed for evaluation in field experiments at three locations in Southern Germany (Stuttgart-Hohenheim, Eckartsweier/Upper Rhine Valley, Frankendorf near Freising), with two replicates in one-row-plots with 20 to 35 plants per row. Because of large differences in vigor, H- and DH-lines were grown in different blocks separated by a mixture of inbred lines differing widely in flowering time. DH lines were machine-harvested, whereas the ears of the H-lines were picked by hand and carefully threshed in the laboratory. Grain yield per plant and TGW were used to calculate the number of grains per plant in the most fertile population (Pop I).

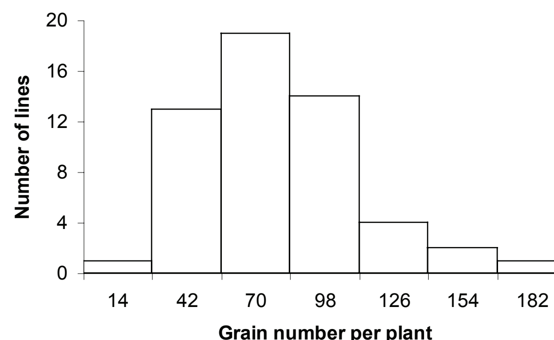
Surprisingly, all H-lines showed a certain degree of female fertility. Yet grain yield per plant was much lower than in the DH lines (Table 1). Among the three H-line populations, one (Pop. I) showed a grain yield several times higher than the remaining two. In all three populations, the maximal grain yield was three to six times higher than the population mean. In Population I, the grain number per plant varied from 25 to 192 (Fig.1A).

Seven H-lines excelled with almost complete seed set. Thousand-grain weight also showed a large range of variation (Fig.1B)

Table 1. Mean, minimum, and maximum values for grain yield per plant [g plant^{-1}] in three F1-derived populations of random haploid and corresponding doubled haploid maize lines averaged across three locations in Southern Germany in 2004 (N = number of lines per population).

Pop.	N	Haploid lines			Doubled haploid lines		
		Mean	Min.	Max.	Mean	Min.	Max.
I	54	12.70	2.93	47.04	61.91	28.19	100.80
II	57	3.45	0.58	9.22	74.55	38.69	121.26
III	58	2.14	0.04	14.57	54.47	12.03	97.26

A



B

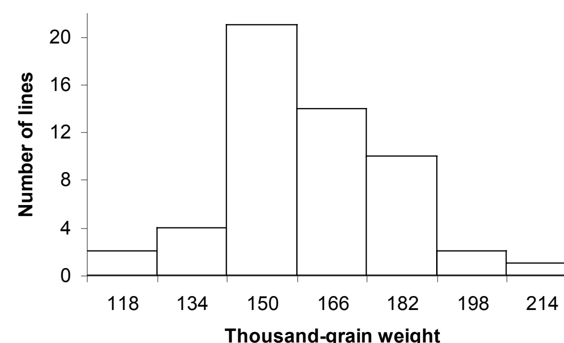


Figure 1. Frequency distribution for (A) grain number per plant and (B) thousand-grain weight [g] of haploid lines in Population I averaged across three locations in Southern Germany in 2004. Figures below the abscissa refer to class means.

averaging across locations to approx. 160g (Table 2). No relationship existed between grain number per plant and TGW, whereas a strong correlation (r = 0.95, P = 0.01) occurred between grain number per plant and grain yield. Seed samples from the most fertile H-lines had full germination capacity (data not shown). Great differences existed between test sites for all three traits measured (Table 2). Remarkably, seed set was more stable across locations than TGW and grain yield.

Table 2. Mean performance of 54 random haploid lines from Population I at three locations in Southern Germany in 2004 (HOH = Hohenheim, EWE = Eckartsweier, FRA = Frankendorf).

Trait	HOH	EWE	FRA	Mean
Grain number per plant	65.32	90.70	84.96	80.32
Thousand-grain weight [g]	171.52	188.01	120.24	159.93
Grain yield [g plant^{-1}]	11.01	17.16	9.92	12.70

Almost all H-plants were absolutely male sterile. Only occasionally were tassels observed with one or a few extruding anthers, some of which released traces of pollen when the anthers were squeezed between one's fingers.

In conclusion, our results revealed great genetic variation in

female fertility among and between H-line populations derived from elite dent single crosses. Several lines showed full seed set with normal-sized, germinable kernels. Thus, the RS scheme proposed by Chalyk and Rotarenco (see above) indeed should work in many breeding materials. Further research is needed to clarify the mechanism leading to fertile egg-cells in haploid maize plants.

TURDA, ROMANIA
Agricultural Research Station

Effects of the *Ht1* or *Ht2* gene in five maize inbred lines on quantitative resistance to *Exserohilum turcicum*
--Has, V; Nagy, E; Has, I

A nonreciprocal factorial set composed of the recurrent parents (T248, T243, Lo3, N4, W153R) and five nearly isogenic lines, each carrying the *Ht1* or *Ht2* gene, were evaluated for quantitative resistance to *Exserohilum turcicum* under artificial inoculation with isolates from Turda and Fundulea and under a very strong natural infection by means of vegetative remains in soil in 2003 and 2004. Field studies were conducted to determine the effectiveness of the *Ht1* or *Ht2* gene in conditioning resistance to *E. turcicum.* Percentage of leaf tissue blighted was significantly lower for hybrids with the *Ht1* gene than for hybrids made from their recurrent parents without the *Ht1* and/or *Ht2* gene when inoculated with isolates "Turda" and "Fundulea", except Lo3 *Ht1* x T248 *Ht2* (Table 1). Hybrids with the *Ht1* gene, except T243 *Ht1* x N4 *Ht1*, had significantly higher grain yield than the same hybrids without the *Ht1* gene under inoculated conditions. The crosses with T243 *Ht1* as maternal parent had significantly fewer lesions and shorter lesions than for its near-isogenic counterpart T243. Significant grain yield differences were detected between: T243 *Ht1* x N4 and T243 x N4, T243 *Ht1* x W153R *Ht1* and T243 x W153R , T243 *Ht1* x Lo 3 *Ht1* and T243 x Lo3, Lo3 *Ht1* x T243 and Lo3 x T243, Lo3 *Ht1* x W153R. Percentage of upright plants was significantly higher for hybrids with the *Ht1* or *Ht2* gene.

When inbred lines were evaluated, reduced differences between original and *Ht1* or *Ht2* versions of the inbred lines T243 and T248 were detected. Conversely, W153R *Ht1*, Lo3 *Ht1* and N4 *Ht1* had higher disease efficiency than W153R, Lo3 and N4 (Table 2).

Results of this study indicate that generally *Ht1* or *Ht2* - converted hybrids are more resistant to *E. turcicum* isolates "Turda" and "Fundulea" than the original versions of these same hybrids. The difference was consistently significant, with the most susceptible hybrid being "*ht ht*". This suggests that the level of quantitative resistance affects the expression of "residual resistance". This study also indicates some maize hybrids and inbred lines with the *Ht1* or *Ht2* gene are more resistant to *E. turcicum* isolates "Turda" and "Fundulea" than near-isogenic hybrids without the *Ht1* or *Ht2* gene.

Table 1. An assessment of northern leaf blight, grain yield and upright plants on eight sets of four maize hybrids homozygous recessive, heterozygous, or homozygous dominant for the *Ht1* and/or *Ht2* gene following inoculation with *E. turcicum* isolates "Turda" and "Fundulea" in 2003 and 2004 at one location in Turda.

Hybrid	Leaf tissue blighted[1]		Yield[2]		Upright plants[3]	
	%	% of standard	q/ha	% of standard	%	% of standard
T243 x T248	27.6	100	61.1	100	16.3	100
T243 x T248 *Ht2*	28.3	103	64.7	106	17.4	107
T243 *Ht1* x T248	16.9	61	69.2	113	16.1	99
T243 *Ht1* x T248 *Ht2*	14.6	53	75.7	124	21.7	133
T243 x N4	32.1	100	58.7	100	15.5	100
T243 x N4 *Ht1*	28.3	88	57.0	97	18.5	119
T243 *Ht1* x N4	18.7	58	81.7	139	20.3	131
T243 *Ht1* x N4 *Ht1*	18.1	56	56.9	97	20.8	134
T243 x W153R	27.1	100	58.5	100	22.7	100
T243 x W153R Ht1	15.4	57	69.1	118	22.0	97
T243 *Ht1* x W153R	10.3	38	67.3	115	26.3	116
T243*Ht1* x W153R *Ht1*	10.3	38	71.1	121	22.7	100
T243 x Lo 3	31.0	100	55.1	100	18.0	100
T243 x Lo 3 *Ht1*	28.1	91	58.2	106	22.1	123
T243 *Ht1* x Lo 3	14.6	47	82.2	149	28.7	159
T243 *Ht1* x Lo 3 *Ht1*	13.7	44	78.9	143	35.0	194
Lo3 x T248	19.6	100	67.1	100	22.0	100
Lo3 x T248 *Ht2*	20.3	104	68.2	102	27.4	125
Lo3 *Ht1* x T248	18.1	92	61.7	92	29.8	135
Lo3 *Ht1* x T248 *Ht2*	20.6	105	73.1	109	32.4	147
Lo3 x T243	29.9	100	55.1	100	24.6	100
Lo3 x T243 *Ht1*	17.7	59	69.3	126	30.3	123
Lo3 *Ht1* x T243	28.8	96	63.4	115	21.7	88
Lo3 *Ht1* x T243 *Ht1*	16.7	56	69.9	127	26.9	107
Lo3 x N4	25.3	100	59.9	100	13.1	100
Lo3 x N4 *Ht1*	16.9	67	67.9	113	25.1	192
Lo3 *Ht1* x N4	22.6	90	66.3	111	18.1	138
Lo3 *Ht1* x N4 *Ht1*	16.3	64	69.2	115	26.7	204
Lo3 x W153R	22.1	100	53.4	100	45.1	100
Lo3 x W153R *Ht1*	11.7	53	54.0	101	65.4	147
Lo3 *Ht1* x W153R	15.2	69	64.9	122	55.7	124
Lo3 *Ht1* x W153R *Ht1*	9.4	42	61.0	114	66.5	147
Ht1 Ht1	26.8	100	58.6	100	22.2	100
Ht1 Ht1	20.8	78	63.5	108	28.5	128
Ht1 Ht1	18.1	68	69.6	119	27.1	122
Ht1 Ht1	15.0	56	69.5	119	30.7	138
LSD (0.05)[4]	2.0		7.6		10.8	

[1] Percentage of leaf tissue blighted 5 wk after the midsilk stage and area affected by the disease is based on visual estimates of leaf tissue blighted in the center row of three-row plots.
[2] Grain yields were adjusted to 15.5% moisture.
[3] Percentage of upright plants were determined at harvest.
[4] Percentage of leaf tissue blighted and upright plants were transformed with the arcsine transformation

Table 2. GCA and SCA effects for resistance to *E. turcicum* (percentage of leaf tissue blighted) under artificial inoculation with isolates from Turda and Fundulea.

Inbred line		T248	T248 *Ht2*	N4	N4 *Ht1*	W153R	W153R *Ht1*	**GCA**	
								ht	**Ht**
T243		-5.5	-0.6	-6.1	1.1	-7.0	-2.7	**9.5**	**4.6**
T243 *Ht1*		3.5	2.1	2.2	6.0	1.7	5.6	**-4.1**	**-5.1**
Lo3		-6.9	-0.9	-6.3	-2.6	-5.4	1.3	**2.9**	**-3.1**
Lo3 *Ht1*		1.4	7.0	2.8	3.1	3.2	3.3	**-0.8**	**-4.0**
GCA	**ht**	**4.2**	**4.9**	**9.3**	**3.2**	**5.2**	**-5.9**	**6.2**	**0.7**
	Ht	**-1.9**	**-1.8**	**1.2**	**-2.2**	**-6.7**	**-9.6**	**-2.3**	**-4.6**

URBANA, ILLINOIS
Maize Genetics Cooperation Stock Center

Additional linkage tests of non-waxy (*Waxy1*) reciprocal translocations involving chromosome 9 at the MGCSC
--Jackson, JD; Stinard, P; Zimmerman, S

Approximately 1 acre each year is devoted to the propagation of the large collection of A-A translocation stocks. In this collection is a series of *Waxy1*-linked translocations that are used for mapping unplaced mutants. Each translocation is maintained in sepa-

rate M14 and W23 inbred backgrounds which are crossed together to produce vigorous hybrids to fill seed requests. Over the years, pedigree and classification problems arose during the propagation of these stocks. We have been able to sort through the problem ones, and can now supply good sources proven by linkage tests to include the correct translocated chromosomes.

Previously we reported the linkage results for some of these stocks (MNL72:79-81; MNL73:86-88; MNL74:67; MNL75:67; MNL76:67-68; MNL77:80; MNL79:48). Below is a summary of additional translocation stocks we have completed testing.

Table 1. ***Wx1*** **T1-9c** (1S.48; 9L.22)

A) The F1 source showed linkage of *wx1* with *P1-ww:*

2 point linkage data for *P1-ww-Wx1* T1-9c
Testcross: [*P1-wr wx1* N x *P1-ww Wx1* T1-9c] x *P1-ww wx1* N wx1

source:87-948 x 951^F1

Region	Phenotype	No.	Totals
0	P1-ww Wx	186	
	P1-wr wx	184	370
1	P1-wr Wx	20	
	P1-ww wx	12	32

% recombination *P1-ww-Wx1*=8.0 ± 1.4

Table 2. ***Wx1*** **T5-9a** (5L.69; 9S.17)

The *Wx1*-marked sources for T5-9a showed no linkage with *v2*. New crossovers were recovered from the *wx1*-marked sources and checked for linkage with *v2*

A) The 4 new M14 crossovers showed no linkage with *v2* and were discarded.

B) One of the 2 new W23 crossovers showed linkage with *v2* and is being converted to M14 to produce vigorous F1s.

2 point linkage data for *v2-Wx1* T5-9a
Testcross: [v2 wx1 N x V2 Wx1 T5-9a] x v2 wx1 N

source: 2002P-388-10 from 2001-1005-6c/o^W23

Region	Phenotype	No.	Totals
0	+ Wx	1504	
	v wx	1542	3046
1	v Wx	47	
	+ wx	58	105

% recombination *v2-Wx1*= 3.3 ± 0.3

Subsequent generations of this new crossover were checked to confirm linkage with *v2.*

source: 2003-1067-1 from 2002P-388-10 from 2001-1005-6c/o^W23

Region	Phenotype	No.	Totals
0	+ Wx	1466	
	v wx	1331	2797
1	v Wx	52	
	+ wx	54	106

% recombination *v2-Wx1*= 3.7 ± 0.3

source: 2003-1067-2 from 2002P-388-10 from 2001-1005-6c/o^W23

Region	Phenotype	No.	Totals
0	+ Wx	1391	
	v wx	1354	2745
1	v Wx	51	
	+ wx	48	99

% recombination *v2-Wx1*= 3.5 ± 0.3

source: 2003-1067-4 from 2002P-388-10 from 2001-1005-6c/o^W23

Region	Phenotype	No.	Totals
0	+ Wx	1213	
	v wx	1264	2477
1	v Wx	79	
	+ wx	47	126

% recombination *v2-Wx1*= 4.8 ± 0.4

source: 2003-1067-5 from 2002P-388-10 from 2001-1005-6c/o^W23

Region	Phenotype	No.	Totals
0	+ Wx	1435	
	v wx	1512	2947
1	v Wx	75	
	+ wx	55	130

% recombination *v2-Wx1*= 4.2 ± 0.4

source: 2003-1067-7 from 2002P-388-10 from 2001-1005-6c/o^W23

Region	Phenotype	No.	Totals
0	+ Wx	1526	
	v wx	1656	3182
1	v Wx	78	
	+ wx	97	175

% recombination *v2-Wx1*=5.2 ± 0.4

source: 2003-1067-10 from 2002P-388-10 from 2001-1005-6c/o^W23

Region	Phenotype	No.	Totals
0	+ Wx	829	
	v wx	848	1677
1	v Wx	39	
	+ wx	47	86

Additional linkage tests of *waxy1* marked reciprocal translocations at the MGCSC

--Jackson, JD; Stinard, P; Zimmerman, S

In the collection of A-A translocation stocks maintained at MGCSC is a series of *waxy1*-linked translocations that are used for mapping unplaced mutants. Also, new *wx1*-linked translocations are being introduced into this series and are in a conversion program to convert each translocation to the inbred backgrounds M14 and W23. These inbred conversions are then crossed together to produce vigorous hybrids to fill seed requests. Over the years, pedigree and classification problems arose during the propagation of these stocks. We have been able to sort through the problem ones, and can now supply good sources proven by linkage tests to include the correct translocated chromosomes. Additional pedigree information on bad sources is available should anyone want to check on sources supplied to them previously by the Stock Center.

Previously we reported the linkage results for some of these stocks (MNL72:81-82; MNL73:88-89; MNL74:67-69; MNL75:68-71; MNL76:65-67; MNL77:79; MNL78:65-66; MNL79:47).

Table 1 contains linkage results for an additional *wx1*-linked translocation stock we have completed testing. This is a stock received from Susan Gabay-Laughnan. She originally obtained it from our 2084B Stock.

Table 1. ***wx1*** **T8-9(043-6)** (8L.17; 9S.34)

A) The new source showed linkage of *wx1* with *v16*:

2 point linkage data for *v16-wx1* T8-9(043-6)
Testcross: [*V16 wx1* T8-9(043-6) x *v16 Wx1* N] x *v16 wx1* N

source:SGL2003-289-1

Region	Phenotype	No.	Totals
0	v Wx	1298	
	+ wx	1856	3154
1	+ Wx	346	
	v wx	345	691

% recombination *v16-wx1*=18.0 ± 0.6

Following is a correction for **Table 6** in Jackson, J and Stinard, P. 1998. MNL72:81-82. The % recombination reported was between *bf2* and *Wx1* not *r1* and *Wx1* as stated.

Table 6. *wx1* T9-10b (9S.13; 10S.40)

A) The M14 sources showed linkage of *wx1* with *bf2*.

2 point linkage data for *bf2*-*wx1* T9-10b
Testcross: [*Bf2 wx1* T9-10b x *bf2 Wx1* N] x *bf2 wx1* N

source:82-116-1^M14

Region	Phenotype	No.	Totals
0	bf Wx	334	
	+ wx	312	646
1	+ Wx	46	
	bf wx	19	65

% recombination ***bf2***-*Wx1*=9.1 ± 1.1

B) The W23 sources showed linkage of *wx1* with *bf2*.

2 point linkage data for *bf2*-*wx1* T9-10b
Testcross: [*Bf2 wx1* T9-10b x *bf2 Wx1* N] x *bf2 wx1* N

source:82-117-1^W23

Region	Phenotype	No.	Totals
0	bf Wx	812	
	+ wx	892	1704
1	+ Wx	40	
	bf wx	39	79

% recombination ***bf2***-*Wx1*=4.4 ± 0.5

Three point linkage data for *Og*-Catlin* places it on 10S

--Jackson, JD

A new dominant yellow stripe stock maps to chromosome 10S near *Og1*. This 'phenotype only' mutation was isolated many years ago from a Dekalb hybrid. Its phenotype is very similar to *Og1*. Crosses were done with the *waxy1*-marked translocations: T9-10b and T8-9(043-6), and to *Wx1*-marked translocation T9-10(4303) to genetically determine its chromosomal location.

The results of three-point linkage tests for *Wx*1, T9-10b, T9-10(4303) and *Og*-Catlin* are presented in Tables 1 and 2. The linkage tests were set up as a modified backcross. Wx and wx kernels from the backcross ears were planted in the field and the resulting plants were scored for yellow stripes and for the presence of the translocation by pollen sterility. The following linkage relationships were established: *Wx1* – 5.6 – T9-10b- 8.4 - *Og*-Catlin* and *Wx1*- 6.4 – T9-10(4303) – 6.4 - *Og*-Catlin*. Crosses were undertaken with *Og1* to determine if *Og*-Catlin* was an allele of *Og1* with little success as *Og1* expression was too weak to follow accurately.

Table 1. Three point linkage data for *Og*-Catlin-Wx1*-T9-10b.

Testcross: *wx1* N *og**x [*Wx1* N *Og*-Catlin* x *wx1* T9-10b *og**]

Region	Phenotype	No.	Totals
0	Wx N str	62	
	wx T gr	63	125
1	Wx T gr	3	
	wx N str	4	7
2	Wx N gr	11	
	wx T str	0	11
1+2	Wx T str	0	
	wx N gr	1	1

% recombination *Wx1*-T =5.6 ±1.9
% recombination T-*Og**=8.3 ± 2.3
% recombination *Wx1*-**Og*=13.2 ± 2.8

Table 2. Three point linkage data for *Og*-Catlin-Wx1*-T9-10(4303).

Testcross: *wx1* N *og**x [*Wx1* T9-10(4303) *og**x *wx1* N *Og*-Catlin*]

Region	Phenotype	No.	Totals
0	Wx T gr	81	
	wx N str	76	157
1	Wx N str	3	
	wx T gr	2	5
2	Wx T str	0	
	wx N gr	5	5
1+2	Wx N gr	4	
	wx T str	2	6

% recombination *Wx1*-T =6.4 ± 1.9
% recombination T-*Og**=6.4 ± 1.9
% recombination *Wx1*-**Og*=12.7 ± 2.5

The results of two-point linkage tests for *Wx1*, T8-9(043-6), Inv9a and *Og*-Catlin* are presented in Tables 3 and 4. The linkage tests were also set up as a modified backcross. Wx and wx kernels from the backcross ears were planted in the field and the resulting plants were scored for yellow stripes. Results indicate no linkage of *Og*-Catlin* with either chromosome 9 or chromosome 8.

Table 3. Two point linkage data for *Og*-Catlin-Wx1*-T8-9(043-6).

Testcross: *og* wx1* N x [*Og*-Catlin Wx1* N x *og* wx1* T8-9(043-6)]

Region	Phenotype	No.	Totals
0	Wx str	40	
	wx gr	48	88
1	Wx gr	45	
	wx str	45	90

% recombination *Wx1**-*Og*= 50.6 ± 3.7

Table 4. Two point linkage data for *Og*-Catlin-Wx1*-Inv9a.

Testcross: *og* wx1* N x [*Og*-Catlin Wx1* N x *og* wx1* Inv9a]

Region	Phenotype	No.	Totals
0	Wx str	16	
	wx gr	52	68
1	Wx gr	50	
	wx str	9	59

% recombination *Wx1**-*Og*= 46.5 ± 4.4

The following is a correction on data previously reported in MNL74:70. This dominant yellow stripe stock also maps to chromosome 10S near *Og1*.

Table 1. Three point linkage data for *Og*-0376-Wx*1-T9-10b.

Testcross: *og* wx1* N x [*Og*-0376 Wx1* N x *og* wx1* T9-10b]

Region	Phenotype	No.	Totals
0	Wx N str	44	
	wx T gr	38	82
1	Wx T gr	4	
	wx N str.	4	8
2	Wx N gr	2	
	wx T str	0	2
1+2	Wx T str	0	
	wx N gr	1	1

% recombination *Wx1*-T =9.7 ± 2.9
% recombination T-*Og**=3.2 ± 1.4
% recombination *Wx1*-**Og*= 12.9 ± 3.5 (originally reported to be 11.8 ± 3.3)

Three mutable and two stable *r1* haplotype-specific aleurone color enhancers map to the same location on chromosome 2

--Stinard, PS

In last year's MNL (79:45), we reported that the mutable *r1* haplotype-specific aleurone color enhancers *Fcu* and *arv-m594* map to the same location and are probably allelic. We obtained additional mapping data for this pair of factors, and tested additional factors for linkage. We tested the mutable factors *Fcu* with *arv-m594*, *arv-m594* with *arv-m694*, and *Fcu* with *arv-m694*. We also tested the stable full color enhancer *Arv-V628#16038* with a full color *Fcu* revertant, *Fcu-R2003-2653-6*. All tests were conducted as follows: Lines homozygous for the two factors, and homozygous for either *r1-g*, or for a responsive *r1* haplotype, were crossed together. The resulting F1's were outcrossed to the re-

sponsive *r1* haplotype *R1-r(Venezuela559-PI302355)* without any factors present. The parental classes and the double factor recombinant class would be expected to have mutable or full colored aleurones, and the recombinant class lacking both factors would be expected to have stable pale or colorless aleurones. Kernels from these crosses were scored for the presence of sectoring in the case of the mutable factors, or full color in the case of the stable factors, and exceptional stable pale or colorless kernels were planted last summer and the resulting plants self-pollinated and outcrossed to *R1-r(Venezuela559-PI302355)* in order to confirm the genotypes of these kernels. Since the only recombinant class that can be detected by these experiments is the class lacking both factors, we doubled the number of kernels in this class in order to account for the double mutant class for the purpose of calculating linkage values. The results are presented below:

***Fcu* with *arv-m594*.** If we combine the data presented last year with the data collected this year, we find no putative crossovers in a population of 3,223 kernels. Thus, we calculate that these two factors are separated by less than 0.06 centiMorgans (cM), and are likely allelic.

***arv-m594* with *arv-m694*.** We isolated four stable pale kernels from a population of 1,620 kernels in this test. However, upon further testing, only two of them proved to be truly stable (not carrying a mutable factor). Thus, we calculate an apparent map distance between these two factors of 0.25 ± 0.12 cM. We could not rule out these kernels being the result of self-contamination by the *R1-r(Venezuela559-PI302355)* tester used as the female in the test crosses, but the plants grown from the exceptional kernels appeared to be vigorous outcross plants and not the result of self-contamination by the tester, which is in a W22 background. We also cannot rule out the possibility that the exceptional kernels represent stable derivatives of the mutable factors and not crossovers. Thus, we conclude that these two factors are either very tightly linked, or more likely, allelic.

***Fcu* with *arv-m694*.** We isolated eight stable pale or colorless kernels from a population of 2,372 kernels in this test. Upon further testing, seven of these proved not to be crossovers, and one did not survive to pollination and could not be tested. Thus we calculate that these two factors are separated by less than 0.08 cM, and are likely allelic.

All three mutable factors were tested for linkage with each other, and few potential crossovers were identified. Those that were identified could actually be stable null derivatives of the mutable factors. We conclude that *Fcu*, *arv-m594*, and *arv-m694* map to virtually the same position, and are likely allelic, if not identical, to each other. For information on the origin of these factors, see Stinard, MNL 79:45.

***Arv-V628#16038* with *Fcu-R2003-2653-6*.** We isolated six stable pale or colorless kernels from a population of 1,887 kernels in this test. Upon further testing, only one of these proved to lack both enhancers. Thus, we calculate a map distance between these two factors of 0.11 ± 0.07 cM. Again, we could not rule out self-contamination by the *R1-r(Venezuela559-PI302355)* tester used as the female in the test crosses, but the plant grown from the exceptional kernel appeared to be a vigorous outcross plant and not the result of self-contamination by the tester. This exceptional kernel could be a true crossover, although other possibilities such as mutation can't be ruled out since these tests were not conducted using detectable flanking markers.

Arv-V628#16038 is a naturally occurring enhancer of aleurone color isolated from the *r1* haplotype *R1-r(Venezuela638#16038)* stock described by Van der Walt and Brink (Genetics 61:677-695, 1969). *Fcu-R2003-2653-6* is a revertant of *Fcu* isolated as described by Stinard (MNL 78:64-65). The potentially identical map location of these two factors raises the interesting question of the origin of the mutable and stable *r1* aleurone color enhancers. Did the mutable factors result from the insertion of a transposable element in one of the naturally occurring stable factors, or did the stable factors arise as a result of reversion or change of state of one of the mutable factors? Only molecular analysis will resolve this question. Since we mapped *Fcu* (and thus the other factors) to a particular segment on the long arm of chromosome 2 (see Stinard, this MNL), we are in the process of trying to tag *Arv-V628#16038* with *Ac* using one of Tom Brutnell's mapped and characterized transposed *Ac* lines. By this technique, we hope to eventually clone and characterize these unique and interesting factors.

The isolation and characterization of *Fcu* germinal revertants, part 3

--Stinard, PS

In the last two newsletters (MNL 78:64-65 and MNL 79:46), we reported on the isolation and characterization of *Fcu* revertants. In 2004, we screened an additional population for revertants. The experiment was set up using the same genetic stocks and techniques as in 2003 (MNL 78:64-65). Out of a population of 11,118 kernels, 29 exceptional full-colored putative revertant kernels were isolated. These 29 kernels were planted this past summer and the resulting plants self-pollinated and outcrossed to the responsive *r1* haplotype tester *R1-r(Venezuela559-PI302355)* in order to test for heritability and test for contamination markers. Of the 29 putative revertants, one proved to be a contaminant, lacking the *y1* and *wx1* markers from the *Fcu* parent; 26 proved not to be heritable, segregating for *Fcu* sectored kernels in both selfs and outcrosses; and 2 proved to be heritable germinal revertants, segregating for full colored stable kernels in both self and outcross, as well as the contamination markers. Thus, the heritable germinal reversion rate for this population is 1.8 X 10^{-4}. Combining these data with the 2003 and 2004 data, we obtained 3 heritable germinal revertants out of a population of 23,430 kernels, for a germinal reversion rate of 1.3 X 10^{-4}. The two germinal revertants isolated in the 2004 experiments have been named *Fcu-R2004-947-5* and *Fcu-R2004-947-10*.

New *inr1* and *inr2* alleles

--Stinard, PS

inr1 and *inr2* are loci with dominant alleles that suppress aleurone color in crosses to specific *r1* haplotypes (Stinard and Sachs, J. Hered. 93:421-428, 2002). To date, several dominant alleles of *inr1* and *inr2* have been isolated from diverse sources (Stinard, MNL 78:62-63 and MNL 79:46). In 2004 (MNL 78:62-63), we reported a putative *inr1* allele that was isolated from an *Fcu* line obtained from Peter Peterson. *Fcu*-induced sectoring and putative *Fcu* revertant events confounded the linkage tests we performed with *Inr1-JD*, but since then, an *Inr*-Fcu* line free of *Fcu* has been

constructed and subjected to linkage analysis. The linkage test was set up as follows: A line homozygous for *Inr1-JD* and the responsive haplotype *R1-Randolph* was crossed to a line homozygous for *Inr*-Fcu* and the responsive haplotype *R1-ch(Stadler)*. F1 kernels were planted, and the resulting plants were crossed by a line homozygous for *R1-Randolph*, without inhibitors. Kernels on the resulting ears were scored for pale vs. full aleurone color, and of 3,712 kernels examined, all were pale. Thus, no full color recombinants lacking both inhibitors were detected, and we calculate the map distance between *Inr1-JD* and *Inr*-Fcu* to be less than 0.054 ± 0.038 centiMorgans. We conclude that these two factors are most likely allelic, and have renamed *Inr*-Fcu* as *Inr1-Fcu*.

As reported previously (Stinard and Sachs, 2002), *Inr1-JD* and *Inr2-JD* were isolated from an open pollinated variety known as John Deere, so named for its green aleurone color. We identified another green aleurone maize variety called Oaxacan Green Corn in the collection of the Abundant Life Seed Foundation of Port Townsend, Washington, obtained seeds of it, and subjected it to the same analysis as John Deere, and found that it, too, carries two *r1* haplotype-specific inhibitors of aleurone color. So far, we have isolated and characterized one of the inhibitors and found it to be most likely an allele of *inr2*. Mapping crosses of this factor with *Inr1-JD* showed independent segregation, but in mapping crosses with *Inr2-JD* carried out in a homozygous *R1-Randolph* background, we found no crossovers in a population of 5,861 kernels, indicating a separation of less than 0.034 ± 0.024 cM between these two factors. We have named this factor *Inr2-OGC*. If the second factor turns out to be allelic to *inr1*, then it seems likely that Oaxacan Green Corn and John Deere are just independently maintained isolates of the same open pollinated variety. Both lines have similar maturity and gross plant morphology (cob color, ear shape and size, tassel branching, etc.).

Five point linkage data for *Fcu* with respect to the chromosome 2 markers *fl1*, *v4*, *w3*, and *ch1*

--Stinard, PS

In MNL 78:63-64 (2004), we reported the placement of *Fcu* to the long arm of chromosome 2 using *wx1* marked reciprocal translocations. In order to further refine the position of *Fcu* on 2L, we conducted a five-point linkage test of *Fcu* with the chromosome 2 markers *fl1*, *v4*, *w3*, and *Ch1*. The linkage test cross and the results are presented in Table 1. A line homozygous for *Fcu* was crossed by a line homozygous for *fl1*, *v4*, and *Ch1*, and segregating for *w3*. Kernels from the resulting cross were planted, and the resulting plants self-pollinated and outcrossed to the *Fcu* responsive *r1* haplotype *R1-r(Venezuela559-PI302355)*. Only those outcrosses of plants carrying the factor *w3* were advanced to the next generation. Kernels from outcross ears were separated into spotted (*Fcu*) and nonspotted (*fcu*) classes, planted in our 2004 Puerto Rico winter and 2005 summer nurseries, and the resulting plants self-pollinated. The resulting ears were scored for the presence of *fl1*, *w3*, and *Ch1*, and scoring was confirmed for *Fcu*. Kernel samples from each ear were planted in sand benches and scored for the presence of *v4*. The results were tabulated, and linkage values were calculated (see Table 1). The linkage order and distances were established as:

fl1 – 13.8 – *v4* – 15.9 – *fcu* – 6.6 – *w3* – 43.9 – *ch1*

The linkage values for *fl1*, *v4*, *w3*, and *ch1* are close to those previously reported on the 1993 genetic map of chromosome 2 (Neuffer et al., Mutants of Maize, Cold Spring Harbor Laboratory Press, 1997), *fl1* – 15 – *v4* – 28 – *w3* – 44 – *ch1*, there being a slight discrepancy in the *v4* – *w3* distance (22.5 cM, our data, vs. 28 cM, genetic map). However, individually reported linkage data from Robertson et al. (Genetics 46:649-662, 1961; 22.7 cM, n = 304; and 23.9, n = 376) and Patterson et al. (MNL 42:44-48, 1968; 24 cM, n = 71) are in close agreement with our data.

Table 1. Five point linkage data for *fl1 v4 fcu w3 ch1*.

Testcross: *R1-r(Venezuela559-PI302355)* X [*Fl1 V4 Fcu W3 ch1* X *fl1 v4 fcu w3 Ch1*]

Region	Phenotype	No.	Totals
0	fl1 v4 fcu w3 Ch1	48	
	Fl1 V4 Fcu W3 ch1	57	105
1	fl1 V4 Fcu W3 ch1	9	
	Fl1 v4 fcu w3 Ch1	7	16
2	fl1 v4 Fcu W3 ch1	10	
	Fl1 V4 fcu w3 Ch1	10	20
3	fl1 v4 fcu W3 ch1	6	
	Fl1 V4 Fcu w3 Ch1	7	13
4	fl1 v4 fcu w3 ch1	42	
	F11 V4 Fcu W3 Ch1	45	87
1 + 2	fl1 V4 fcu w3 Ch1	2	
	Fl1 v4 Fcu W3 ch1	3	5
1 + 3	fl1 V4 Fcu w3 Ch1	1	
	Fl1 v4 fcu W3 ch1	1	2
1 + 4	fl1 V4 Fcu W3 Ch1	11	
	Fl1 v4 fcu w3 ch1	6	17
2 + 3	fl1 v4 Fcu w3 Ch1	0	
	Fl1 V4 fcu W3 ch1	1	1
2 + 4	fl1 v4 Fcu W3 Ch1	7	
	Fl1 V4 fcu w3 ch1	13	20
3 + 4	fl1 v4 fcu W3 Ch1	2	
	Fl1 V4 Fcu w3 ch1	1	3
Total (n)			289
No triple or quadruple crossovers			

Map distance *fl1* - *v4* = 13.8 ± 2.0 cM
Map distance *v4* - *fcu* = 15.9 ± 2.2 cM
Map distance *fcu* - *w3* = 6.6 ± 1.5 cM
Map distance *w3* - *ch1* = 43.9 ± 3.0 cM

Near colorless (*Nc*) enhancing effects of the *Fcu/Arv r1* haplotype-specific aleurone color enhancers

--Stinard, PS

Members of the *Fcu* and *Arv* class of *r1* haplotype-specific aleurone color modifiers enhance aleurone color in crosses to receptive *r1* haplotypes such as *r1-cu* and *R1-r(Venezuela559-PI302355)* (Stinard, MNL 77:77-79, 2003). So far, all responsive haplotypes surveyed seem to be members of the class of *r1* haplotypes that have seed color components containing inverted repeats of coding sequences flanking rearranged *Doppia* transposable element sequences (Walker and Panavas, Genetics 159:1201-1215, 2001), although not all haplotypes tested have been subjected to molecular analysis.

Some derivatives of *R1-st* include haplotypes that carry genes called *Nc* (*Near colorless*) that consist of coding sequences carrying *Doppia* sequences in their promoter region (Matzke et al., Trends Plant Sci. 1:382-388, 1996). In order to test *Fcu/Arv* for ability to enhance aleurone color expression of *Nc* genes, a line

homozygous for the stable enhancer *Arv-V628#16038* and the colorless aleurone *r1* haplotype *r1-g(Stadler)* was crossed to a line homozygous for *r1-sc:m6*, a derivative of *R1-sc:134* that carries a single functional *Nc* gene (Eggleston et al., Genetics 141:347-360, 1995) and has its main seed color component, *Sc*, inactivated by a *Ds* transposable element insertion (Jerry Kermicle, personal communication). Kernels from this cross had a lightly mottled phenotype, whereas the *r1-sc:m6* parental line without enhancers had virtually colorless aleurone. (*Ac* is not present in these lines.) Thus, *Arv-V628#16038* appears to enhance *Nc* expression. Kernels from the F1 were planted this past summer, and the resulting plants self-pollinated. The resulting ears segregated for dark and light mottled kernels, perhaps reflecting different dosages of the enhancer, *Arv-V628#16038*, and the *Nc* receptor haplotype, *r1-sc:m6*. Attempts will be made to isolate lines homozygous for both enhancer and receptor.

Crosses of *Arv-V628#16038* were also made to the *Nc* line carrying the haplotype *r1-g(Nc)3-5*, which is a derivative of *R1-st* that carries an Sc component inactivated by a transposition-defective *I-R* element (Kermicle, Genetics 107:489-500, 1984), and likely carries three *Nc* genes, as it was derived directly from *R1-st*, which also carries three *Nc* genes (Eggleston et al., 1995). All kernels from such crosses had virtually colorless aleurone, but such a result is not unexpected since multiple copies of *Nc* genes *in cis* seem to have an inhibitory effect on *Nc* expression (Eggleston et al., 1995), and therefore any enhancing effect of *Arv-V628#16038* on *Nc* might go undetected.

Factors known to enhance *Nc* expression are present in open-pollinated populations (Jerry Kermicle, personal communication). A stock homozygous for one such *Nc* enhancer isolated from the land race Zapalote Chico, and homozygous for *r1-g(Stadler)*, was obtained from Jerry Kermicle. We outcrossed this stock to *r1-sc:m6*, *r1-g(Nc)3-5*, and to *R1-r(Venezuela559-PI302355)*. Crosses to *r1-sc:m6* produced a light mottled phenotype, and crosses to *R1-r(Venezuela559-PI302355)* produced full-colored kernels. On the other hand, crosses to *r1-g(Nc)3-5* produced virtually colorless kernels. Thus, this *Nc* enhancer seems to behave similarly to *Arv-V628#16038*. In order to determine whether these factors might be closely linked or allelic, we crossed the *Nc* enhancer to *Arv-V628#16038*, and hope to have the results of mapping tests completed by the end of next summer.

It remains an open question as to whether the *Doppia* sequences associated with coding sequences in responsive haplotypes have anything to do with the observed response to enhancers. It remains an attractive hypothesis, but only molecular analysis will resolve this question.

Mapping data for *arv-m694*, *Fcu-R2003-2653-6*, and *Arv-V628#16038* with respect to *wx1* T2-9d

--Stinard, PS

In 2004 (MNL 78:63-64), we reported the results of linkage tests of the mutable *r1* haplotype-specific enhancers of aleurone color *Fcu* and *arv-m594* with respect to *wx1* in a set of *wx1* marked reciprocal translocations. Tightest linkage with *wx1* was obtained for both factors with the translocation *wx1* T2-9d (breakpoints 2L.83; 9L.27). Since then, linkage data with respect to the chromosome 2 markers *fl1*, *v4*, *w3*, and *Ch1* have further refined the position of *Fcu* on 2L (Stinard; this MNL), and the factors *Fcu*, *arv-m594*, *arv-m694*, *Arv-V628#16038*, and *Fcu-R2003-2653-6* have been found to map to the same position (Stinard; this MNL). In parallel, linkage crosses of *arv-m694*, *Fcu-R2003-2653-6*, and *Arv-V628#16038* were performed with *wx1* T2-9d, and the results are reported below.

Linkage crosses were set up as indicated in Tables 1 (*arv-m694*), 2 (*Fcu-R2003-2653-6*), and 3 (*Arv-V628#16038*). Lines homozygous for enhancers were crossed to *wx1* T2-9d, and the resulting F1's were outcrossed as males to tester lines homozygous for *wx1* and the reporter haplotype *R1-r(Venezuela559-PI302355)*. Kernels from the test cross ears were scored for waxy (wx) *vs*. starchy (Wx) endosperm and sectored (arv-m) or full-colored (Fcu-R or Arv) vs. stable pale (arv or fcu) aleurone. The results and linkage values are reported in Tables 1, 2, and 3.

Table 1. Two point linkage data for *wx1* and *arv-m694* in crosses involving T2-9d. Data from four ears.

Testcross: *wx1* N *arv* *R1-r(Venezuela559-PI302355)* X [*wx1* T *arv* *r1* X *Wx1* N *arv-m694* *R1-r(Venezuela694#16037)*]

Region	Phenotype	No.	Totals
0	Wx arv-m	265	
	wx arv	228	493
1	wx arv-m	49	
	Wx arv	45	94
n			587

Map distance *wx1* – *arv-m694* = 16.0 ± 1.5 cM

Table 2. Two point linkage data for *wx1* and *Fcu-R2003-2653-6* in crosses involving T2-9d. Data from three ears.

Testcross: *wx1* N *fcu* *R1-r(Venezuela559-PI302355)* X [*wx1* T *fcu* *r1* X *Wx1* N *Fcu-R2003-2653-6* *R1-r(Venezuela559-PI302355)*]

Region	Phenotype	No.	Totals
0	Wx Fcu-R	153	
	wx fcu	155	308
1	wx Fcu-R	15	
	Wx fcu	25	40
n			348

Map distance *wx1* – *Fcu-R2003-2653-6* = 11.5 ± 1.7 cM

Table 3. Two point linkage data for *wx1* and *Arv-V628#16038* in crosses involving T2-9d. Data from six ears.

Testcross: *wx1* N *arv* *R1-r(Venezuela559-PI302355)* X [*wx1* T *arv* *r1* X *Wx1* N *Arv-V628#16038* *r1-g (Stadler)*]

Region	Phenotype	No.	Totals
0	Wx Arv	356	
	wx arv	360	716
1	wx Arv	47	
	Wx arv	79	126
n			842

Map distance *wx1* – *Arv-V628#16038* = 15.0 ± 1.2 cM

All three enhancers showed linkage with *wx1* in crosses involving T2-9d, although the map distances varied somewhat. The values with respect to *arv-m694* (16.0 ± 1.5 cM), *Fcu-R2003-2653-6* (11.5 ± 1.7 cM), and *Arv-V628#16038* (15.0 ± 1.2 cM) maybe be directly compared with those previously reported for *Fcu* (13.6 ± 0.9 cM) and *arv-m594* (9.4 ± 0.8 cM; MNL 78:63-64). All five factors show little if any recombination with each other (Stinard, this MNL). However, discrepancies between linkage values

with respect to *wx1* marked translocations are not unusual (EB Patterson, Ph.D. Dissertation, 1952) and could be due to differences in genetic background or environmental factors.

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Flow cytometry analysis of DNA content in diploid and autotetraploid maize with B chromosomes

--Carvalho, CR; Saraiva, LS; Mendonça, MAC

Flow cytometry methodology was used to study the DNA content in maize diploids and tetraploids, with and without B chromosomes. The diploid with B chromosome genomes had a 22.4 % increase in DNA over the diploid without B chromosomes, and the autotetraploid with B chromosomes showed only a 3.2% increase in DNA content as compared with the autotetraploid without B chromosomes. Flow cytometry methodology resulted in differentiated histogram peaks to resolve the presence at one B chromosome level, being a useful and highly sensitive methodology for comparative analysis of DNA content differences in maize genomes, as well as for plant B chromosome evolution studies.

Diploid (2x) and diploid plus B chromosome (2x+B) seeds of Black Mexican Sweet Corn, autotetraploid (4x) of N102A, and autotetraploid plus B chromosome (4x+B) of N102E stocks, were kindly supplied from the Maize Genetics Cooperation Stock Center, University of Illinois, USA. As internal standard (5.43 pg-DNA), maize diploid seeds (2x) cv. CE–777 were kindly supplied by Dr. Jaroslav Dolezel (Lab. Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Czech Republic).

Fresh leaves (0.5 cm^2) were chopped with a razor blade in a petri dish with 0.5 ml nucleus-isolation buffer (Partec ©). After 1 min, the suspension was passed through a 50 μm mesh nylon filter with 2 ml of 4'-6-diamidino–2 phenylindole (DAPI) solution (Cy-StainUV, Partec®) and stored in the dark for 3 min. Test and standard nuclear materials were processed using a PAS-Partec ® cytometer, previously calibrated and tuned to an excitation wavelength band for DAPI, and analyzed by FlowMax® software. The DNA picogram values were calculated by conversion of data from G_1 peak channels. For each sample, approximately 5000 nuclei were analyzed and those with a coefficient of variation (CV) higher than 3.0 were discarded.

The methodology resulted in a high-resolution histogram (Fig. 1) showing the 2x plant G_1 peak running at channel 100 and the 2x+B plant at channel 122.4. The 5.37 pg DNA value of the 2x plant was determined previously in this work (histogram not shown) using a maize DNA internal standard (5.43 pg). The 2x plant (5.37 pg) was then used as a parameter to calculate DNA content of 6.57 pg in the 2x+B plant. These data show the contribution of maize B chromosomes to the DNA amount to be 1.2 pg (22.4%) in this Black Mexican genome.

The 4x plant had the DNA value of 10.48 pg, determined previously in this work using maize internal standard (histogram not shown). The histogram for 4x and 4x+B plants shows G_1 peaks at channels 200 and 206.4, corresponding to 10.48 pg and 10.82 pg DNA values, respectively (Fig. 2). This genome size difference in tetraploids corresponds to 3.2% due to B chromosome presence in

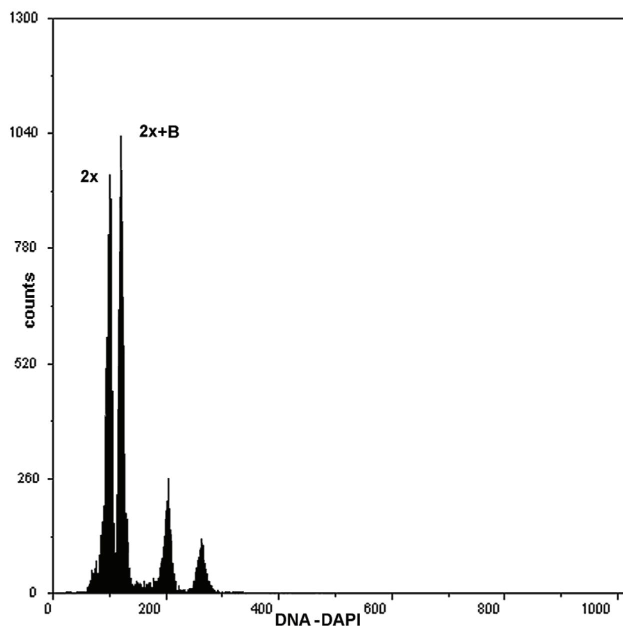

Figure 1. DNA histogram of the nuclei DAPI stained from leaves of diploid (2x) and diploid plus B (2x+B) chromosomes of Black Mexican Sweet Corn. The G_1 peak of the 2x was set to channel 100. The G_1 peak of nuclei from 2x+B was at channel 122.4, corresponding to 5.37 and 6.57 pg DNA values, respectively.

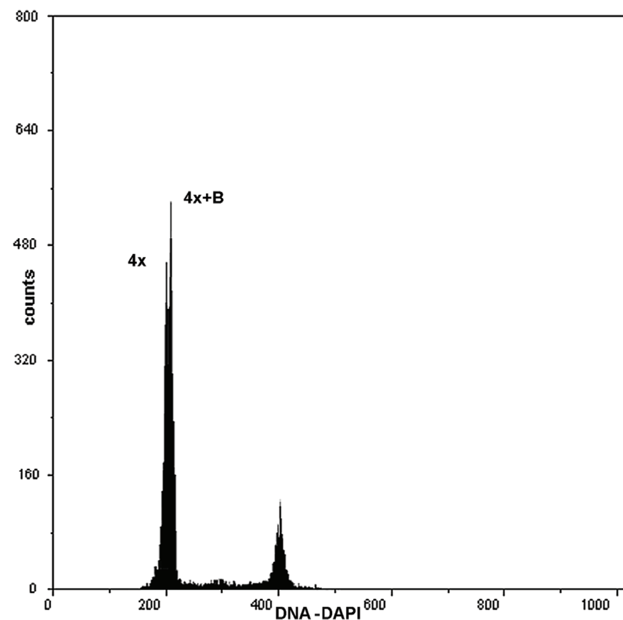

Figure 2. DNA histogram of the nuclei DAPI stained from leaves of autotetraploid (4x) and autotetraploid plus B (4x+B) chromosomes of N102A and N102E maize stocks, respectively. The peak G_1 of 4x plant nuclei running at channel 200 (10.48 pg DNA) and very close to the G_1 peak of 4x+B at channel 206.4 (10.82 pg DNA).

the N102E stock.

Ayonoadu and Rees (Heredity 27:365-383,1971) studied the genome size in individuals without B and with 8B chromosomes in the diploid Black Mexican Sweet corn line, and they calculated that each additional B increases the DNA content by 5%. In the present study, the Black Mexican Sweet sample shows 4B chromosomes, as described by Carvalho et al (MNL 77:80, 2003). According to this data, the genome size difference analyzed by flow

cytometry shows a 22.4% increase in DNA amount for the 2x+B in relation to the 2x, giving an average of 5.6% for each B chromosome. The 3.2% DNA content due to the B chromosome in the 4x+B N102E stock suggests the presence of only one B chromosome.

This methodology was able to detect DNA content differences in genome sizes with B chromosomes at the diploid and tetraploid levels. It was concluded that each B chromosome increased DNA amount approximately 5.6% in 2x+B Black Mexican Sweet, and only 3.2% in 4x+B N102E samples.

Flow cytometry methodology resulted in differentiated histogram peaks to resolve the presence at one B chromosome level, being a useful and highly sensitive methodology for comparative analysis of DNA content differences in maize genomes, as well as for plant B chromosome evolution studies.

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3,216 seed samples have been supplied in response to 290 requests, for 2005. A total of 96 requests were received from 28 foreign countries. More than 90% of our requests were received by electronic mail or through our order form on the World Wide Web. Popular stock requests include the IBM RIL mapping populations, Hi-II lines, *ig1* lines, transposable element lines, Maize Gene Discovery Project lines, and Chromatin stocks.

Approximately 10 acres of nursery were grown this summer at the Crop Sciences Research & Education Center located at the University of Illinois. Favorable weather in the early spring allowed the timely planting of our first crossing nursery. However, dry weather set in, and tender young seedlings in our second crossing nursery were devoured by red-winged blackbirds apparently seeking a source of water. Much of our second crossing nursery will have to be replanted next summer. Growing conditions were generally good, but we had to supplement scant rainfall with irrigation during the first part of the summer. Moderate temperatures and low plant stress resulted in a good pollination season.

Special plantings were made of several categories of stocks:

1. In the 'Phenotype Only' collection, we have made available an additional 250 stocks in 2005.

2. Plantings were also made from donated stocks from the collections of Alice Barkan (photosynthetic mutants), Ed Coe (*bu1*, *w3*, and *nec3* alleles), Hugo Dooner (transposed *Ac* lines), Jerry Kermicle (various *r1* alleles), Robert Lambert (defective kernel mutants), Bruce May and Rob Martienssen (*mn** mutants from the MTM project), Gerry Neuffer (EMS-induced mutants), Pat Schnable (glossy mutants), Margaret Smith (male sterile cytoplasm lines), Keith Slotkin (*Mu killer*), and others. We expect to receive additional accessions of stocks from maize geneticists within the upcoming year.

3. We conducted allelism tests of several categories of mutants with similar phenotype or chromosome location. We found additional alleles of *inhibitor of r1 aleurone color2* (*inr2*), *pink scutellum1*, *spotted1*, *viviparous9*, *ramosa1* and *white14*. We plan to test additional members of the viviparous, spotted leaf, and pale green plant mutants. In this manner, we hope to move more stocks from our vast collection of unplaced uncharacterized mutants into the main collection.

4. We further characterized the *Fcu* system of *r1* aleurone color enhancers. We refined the map position of *Fcu* on the long arm of chromosome 2 through the collection of five point linkage data, and set up crosses to transposon tag *Fcu* using one of Tom Brutnell's transposed *Ac* lines. We are collecting and characterizing additional alleles of *Fcu* and other *r1* aleurone color enhancers.

5. Two acres were devoted to the propagation of the large collection of cytological variants, including A-A translocation stocks and inversions. In this collection is a series of *waxy1*-marked translocations that are used for mapping unplaced mutants. Over the years, pedigree and classification problems arose during the propagation of these stocks. We can now supply good sources proven by linkage tests to include the correct translocated chromosomes. Additional translocation stocks from this series were tested this last year. Results of these tests will be reported in the next issue of the Maize Genetics Cooperation Newsletter. We have received *wx1*, *su1, o2* and *y1* marked translocations from W. R. Findley and *wx*1 marked stocks from Don Robertson. Approximately 30 of these uniquely marked translocation stocks have been added to our collection. Several others will be added as they are increased. Those marked with *wx1* are being checked by linkage tests as we did for the main series of *waxy1*-marked translocations.

6. Stocks produced from the NSF project "Regulation of Maize Inflorescence Architecture" (see: https://www.fastlane.nsf.gov/servlet/showaward?award=0110189) were grown this summer. Families that were observed in 2004 to segregate mutations were selected to be increased in the nursery. These increases help to confirm the presence of the mutation and maintain adequate seed stock to fill future requests.

We continue to grow a winter nursery of 0.5 acres at the Illinois Crop Improvement Association's facilities in Juana Díaz, Puerto Rico. We had an excellent winter crop last year, and all indications are that the crop will perform well this year. We plan to continue growing our winter nurseries at this location.

We have received 2,668 additional EMS lines from various inbred backgrounds produced by Dr. Gerry Neuffer (Regulation of Inflorescence Architecture in Maize project). There are sufficient seed for all of these for distribution. In addition to the EMS lines, we have received an additional 21 lines of Functional Genomics of Chromatin project stocks from Karen McGinnis.

We selected 1,620 lines of materials from Dr. Gerry Neuffer's EMS material that were screened for ear and kernel mutations in the lab, for placement in observation fields on the University of Illinois Crop Science Research facility for observation of seedling and adult plant mutations (during our annual mutant hunt). In addition to these lines, 1,982 lines of MTM material from Dr. Rob Martienssen were also planted this year and observed for phenotype variation. Many visitors from surrounding universities came throughout the summer to walk the fields and search for unique mutations. We plan to have another mutant hunt next summer.

Our IT specialist, Jason Carter, has completed the second version of our curation tools, and we are currently using these tools to maintain data for our collection. These tools now input our public stock data directly into MaizeGDB, to give maize scientists access to up-to-date information about our collection. It is hoped that these curation tools will provide the foundation for the development of more advanced curation tool options for MaizeGDB data curators. Jason has left us to join the Peace Corps, and Josh Tolbert joined us in December. Next year, we plan to develop even more custom software to facilitate the search and display of stock pedigree information, as well as to streamline the process of filling stock requests.

The new greenhouse space in Urbana is almost ready and new seed storage space (which will double our capacity) is presently being designed.

Marty Sachs Philip Stinard Janet Day Jackson Shane Zimmerman Jason Carter/Josh Tolbert

ADDITIONS TO OUR CATALOG OF STOCKS SINCE MNL79

(For a complete list of our stocks, see: http://maizegdb.org/cgi-bin/stockcatalog.cgi)

CHROMOSOME 1 MARKERS
114H ts2-N2491

CHROMOSOME 2 MARKERS
213K w3-N1907

CHROMOSOME 3 MARKERS
304J wlu8-N1906

CHROMOSOME 5 MARKERS
511I a2 ga2-Rhoades

CHROMOSOME 6 MARKERS
608M tdy1-shadow

CHROMOSOME 7 MARKERS
702J bu1
704G gl1-N489B
711H ra1-N408E

CHROMOSOME 9 MARKERS
905J wx1-0601-Alexander

CHROMOSOME 10 MARKERS
X17A R1-sc
X18F g1 R1-st Mst1 o7

CHROMDB STOCKS
3201-01.1-T-MCG3348.07
3201-23.1 T-MCG4361.13
3201-27.2 T-MCG5104.05
3201-35.2 T-MCG5812.05
3201-36 T-MCG3331.08
3201-36.1 T-MCG3331.09
3201-37 T-MCG4301.5
3201-38 T-MCG4716.03
3201-39 T-MCG5801.14
3201-39.1 T-MCG5801.18
3201-39.2 T-MCG5801.26
3201-40 T-MCG5211.06
3201-40.1 T-MCG5211.12
3201-41 T-MCG6123.15

UNPLACED GENES
U139I sur1-B90
U140J zn2-4-6(4461)

ALIEN ADDITION
T940H Tr5

CYTOPLASMIC-STERILE / RESTORER
C736BA Ky21 (J) Restored; cms-J Rf1 Rf2 Rf3 RfC

TOOLKIT
T3212H tac917.12

INVERSION
I143E Inv1f (1S.85; 1L.56)
I144A Inv1g (1S.41; 1L.35)
I144B Inv1h (1L.70; 1L.87)
I144C Inv1l (1S.82; 1L.46)
I144D Inv1m (1S.81; 1L.10)
I243B Inv2h (2L.13; 2L.51)
I243D Inv2e(3718) (2S.44; 2S.84)
I243E Inv2c (2L.1; 2L.6)
I244A Inv2g (2S.88; 2L.50)
I244B Inv2l (2S.38; 2L.54)
I244C Inv2o (2S.73; 2L.70)
I344B Inv3d (3S.72; L.42)
I344C Inv3e (3S.46; L.80)
I443B Inv4c (4S.89; 4L.62)
I443F Inv4h (4L.16; 4L.56)
I443G Inv4i (4L.19; 4L.66)
I443H Inv4j (4L.24; 4L.66)
I444B Inv4l
I543A Inv4e (4L.16; 4L.81)
I543C Inv5b (5S.80; 5L.91)
I543D Inv5d (5S.42; 5L.63)
I543F Inv5f (5S.67; 5L.69)
I643A Inv5h
I643B Inv5i
I643C Inv6b (6S.38; 6L.92)
I843B Inv7b (7S.32; 7L.30)
I843C Inv7e (7S.89; 7L.93)
I843D Inv8b (8L.10; 8L.42)
I843E Inv8c (8S.11; 8S.98)
IX43B Inv9b (9S.05; 9L.87)
IX43C Inv9c (9S.10; 9L.67)
IX43D Inv10a (10S.57; 10L.86)

PHENOTYPE ONLY

adherent leaf
3610F ad*-N705B

albescent
3611C al*-N2362B

albino seedling
3511A w*-N1126B

anthocyanin
3611F atc*-N2368
3611G atc*-N2486

bleached leaf
3612I blh*-N2381
3612K Blh*-N2472

defective kernel
3703A de*-N940
3705H de*-N1152
3705L de*-N1174B
3706A de*-N1192

dented kernel
3606C dnt*-N1116

dwarf plant
4406O d*-N2363B
4407A d*-N2367A
4407B d*-N2371
4407D d*-N2374
4407G d*-N2480
4407H d*-N2498

etched endosperm
3802B et*-N557
3803M et*-N956A
3804G et*-N1187B

golden plant
6008B g*-PI254852

green striped leaf
4009G gs*-N163B
4010E gs*-N2382
4010F gs*-N2405

gritty leaf
4010I gtl*-N2488

high chlorophyll fluorescence
4011B hcf*-N1490B

lesion
3908D les*-N1395C
3908O les*-N2333A
3908P les*-N2363A
3909B Les*-N2397
3909C Les*-N2418
3909E les*-N2502
3909F Les*-NA1176
3909G les*-NA467

lethal
3811M ll*-N2469

male sterile
4012C ms*-N2387B
4012J ms*-N2492B
4012K ms*-N2497A
4012L ms*-N2499

many tillers
4209D Tlr*-N2444

miniature kernel
138-22 mn*-MTM4513
138-35 mn*-MTM4746
238-13 mn*-MTM5341
238-25 mn*-MTM5598
238-29 mn*-MTM5622
238-37 mn*-MTM5806
338-03 mn*-MTM5921
338-14 mn*-MTM6355
338-26 mn*-MTM6975
438-16 mn*-MTM12410
438-42 mn*-MTM15916
638-51 mn*-MTM22311

nana plant
4407J na*-N2366

narrow leaf
3911I nl*-N799B
3911M nl*-N2360B

necrotic leaf
4104KA nec*-N1309B
4104T nec*-N2494
6106Q yg-nec*-92-1260-79

opaque endosperm
3904K o*-N1111A
3905E o*-N1174A
3906G o*-N1297A

pale green plant
4205N pg*-N498
4304I pg*-N2380
4304M pg*-N2406
4305C pgsm*-N2414

pale green seedling
4203F pg*-N277
4304H pg*-N2369
4304J pg*-N2383A
4304K pg*-N2387A
4304N Pg*-N2447
4304O Pg*-N2473

pale midrib
4305E Pm*-N2443

pale pale green leaf
4306C ppg*-N1986

patched leaf
4105I ptc*-N1076B
4105J ptc*-N2352B

pigmy plant
4407K py*-N519

red auricle
5809A rau*-6522

salmon silks
5809A rau*-6522

semidwarf
4408J sdw*-N2391A
4408K sdw*-N2392

small kernel
4002D smk*-N1026A

small plant
4410N smp*-N1324B
4411F smp*-N2372

streaked leaf
3711B stk*-N1115B

striate leaf
3709C sr*-N2395
3709D sr*-N2400

tassel seed
4012U ts*-N2490

torn leaf
4107V trn*-N2370A

tube leaf
3912D tbl*-N1113B

unbranched tassel
4012Q sgsp*-N2412

virescent seedling
4510L v*-N1809

white luteus seedling
4112A wl*-N938B

white sheath
4210E Ws*-N2454

white stripe leaf
4211O str*-N1111B

wilted plant
4209E wi*-N811A
4209J wi*-N2419

yellow green leaf
4308C Yg*-N1586
4309H yg*-N2492A
4309I yg*-N2493

yellow streak leaf
3812Q ysk*-N2385
3812S ysk*-N2481

yellow stripe leaf
3812G ys*-N634B
3812M ys*-N2361
3812O ys*-N2398

zebra necrotic leaf
3712K zn*-N2399A
3712M zn*-N2497B

V. MAIZE GENOME DATABASE

www.maizegdb.org

Reverse Genetics: TILLING, *Ac* and *Ds* Toolkits, RNAi

Integration of the Maize TILLING Project's mutant information into MaizeGDB has been modeled, and will soon be available. Please visit http://genome.purdue.edu/maizetilling for projected release dates.

Information about stocks available from the Stock Center representing *Ds* or *Ac* tags from Hugo Dooner's and Tom Brutnell's projects is regularly updated. RNAi transgenic stocks available at the Stock Center are incorporated, using information provided by the Maize Chromatin Project and linked to targeted genes and constructs within MaizeGDB; links are also provided to the Chromatin Consortium database (http://www.chromdb.org). Query on the Stock page http://www.maizegdb.org/stock.php for stocks of type="Toolkit" and the focus linkage group of interest to retrieve the *Ac* and *Ds* insertion set; query for stocks of type="ChromDB" will retrieve RNAi stocks.

Sequences

Updated raw and assembled sequence sets are loaded into MaizeGDB on the first Tuesday of each month from a dataset prepared by Volker Brendel's PlantGDB project group (Dong et al., Plant Physiol. 139:610-618, 2005). The sequence set includes all maize sequences available at GenBank as well as PlantGDB's transcript contigs which are called PUTs (for "PlantGDB-assembled Unique Transcript Fragment") and GSS contigs (called GSStucs for "Genome Survey Sequence tentatively unique contigs"). For access to the most up-to-date sequence sets, visit PlantGDB (http://www.plantgdb.org) where the sequence set is updated daily. To gain access to sequences and their related data (e.g., map positions, probe information, etc.) visit MaizeGDB. Note that reciprocal linkages between both sites are present at both sites, enabling ease of navigation no matter where you start your sequence search.

Genetic Maps

The updated community IBM map (cIBM 2005) based on the IBM-94 mapping population includes some 1930 loci, where 580 now serve as the framework in the MapMaker computation, compared to 250 framework loci in previous versions of the cIBM maps. This framework was developed in collaboration with Ed Coe and is based on the framework used by Georgia Davis for quantitative trait mappings. Most of the new loci were incorporated from the Genoplante EST-based RFLP mapping (see also maps IBM GNP2004; Falque et al., Genetics 170:1957-1966, 2005). Other loci were mapped from data released by the Maize Chromatin Project (Karen Cone); the Maize Mapping Project (Mike McMullen); and the *Ac* Mutagensis Project (Tom Brutnell). The cIBM maps may be viewed, along with supporting data including map scores, probe details, etc., at MaizeGDB.

The new neighbors map (IBM2 neighbors 2005) now includes some 35,000 probed sites, most of which are related to public ESTs. In addition to the previously included genetic maps, this consensus map computation incorporates order information from several new maps,including Pat Schnable's IDP maps; RFLP maps from Genoplante (Falque et al., 2005); the Cornell University (CU) maps; the MITEs maps; and frame work loci on the Genetic 2005 maps, which encompass the UMC 98 RFLP maps in order to permit incorporation into neighbors. In addition, this build includes any locus ordered onto anchored BAC contigs in the maize FPC product (http://www.arizona.genome.edu). Only loci identified by at least 2 hits by a probe are included (see maps IBM2 FPC0507); probes represented include overgos, RFLPs, and SSRs. The IBM2 FPC0507 maps were built in collaboration with Mike McMullen as a part of the Maize Diversity Project.

Plant Ontology Curation

The Plant Ontology Consortium (http://www.plantontology.org) has developed over the past few years a logical structure for describing plant anatomy, development and growth. While the initial emphasis has been on 3 plants: *Arabidopsis*, rice, and maize, support for several other angiosperm crop plants is now included. At MaizeGDB, anatomical aspects of phenotypes have been associated with Plant Ontology accessions and the phenotype-inferred associations to loci, stocks and alleles have been supplied to the Plant Ontology database (http://www.plantontology.org. Curation tools for annotation of other types of gene expression are under development to add to associations inferred by mutant or trait phenotype.

Community Curation at MaizeGDB

A quantitative trait experiment module has added to the curation tool suite at MaizeGDB (Schaeffer et al., in preparation). This module builds on the earlier tools used at MaizeDB (Byrne et al., J. Agric. Genomics 1:1-11, 1995) and adds many automated and quality control features. For example, much of the nomenclature of objects (maps, QTL, alleles, and panels of stocks) are now auto-computed from symbols assigned to the trait and parental germplasm. Similar to the case for previous community curation tools, the record may be updated by the contributor up until the time it is considered ready for the monthly release. Changes made to data that have been released should be made by communicating with a staff curator. We encourage persons with publications in press to submit their data to MaizeGDB, beginning with the contact form provided on each page at MaizeGDB. Refer to http://www.maizegdb.org/data_contribution.php for more information about data submission to MaizeGDB.

How You Can Help MaizeGDB to Succeed

As you know, MaizeGDB is a 'model organism database' and is the community of maize researchers' repository for genomic and genetic data pertaining to our favorite plant, *Zea mays* ssp. *mays*. At the Community Forum session during the Maize Genetics Conference this past year, the statement that MaizeGDB needs to evolve into a more TAIR-like resource (see http://www.arabidopsis.org) was made, and a desire for the community database to improve was communicated by many attendees, both during and after that session (minutes from the Community Forum can be accessed at http://www.maizegdb.org/maize_meeting/2006/ and in this MNL, pp. 111-113). Subsequently, the Maize Genetics Executive Committee surveyed the community to find out what issues were of the most importance to the community of maize geneticists. The survey's full report can be accessed at http://www.maizegdb.org/SurveyResults06.doc and in this MNL, pp. 114-117. The top three "General Community Needs" identified were:

1. High quality maize genome sequence annotation.
2. Improved maize reverse genetics resources that allow investigators to move from sequence to seed.
3. An improved maize database that allows investigators to move seamlessly between multiple genomic datasets and expression analysis.

Improving the MaizeGDB addresses needs one and two, and the desire for an improved database is stated per se as need three. Help us to meet your needs by voicing this concern! To find out how to help, read "Plant Biology Database s: A Needs Assessment", an advisory whitepaper to the NSF and USDA, which can be accessed at http://www.maizegdb.org/PDBNeeds.pdf, and contact us directly at mgdb@iastate.edu. For an abbreviated version of the Advisory Whitepaper's recommendations, see Stein et al. (The Scientist 20(4):24-25, 2006).

Acknowledgements

We thank MaizeGDB's interface developer and bioinformatics engineer Trent Seigfried and database administrator Darwin Campbell for supporting the curation efforts described here. We are grateful for assistance from the MaizeGDB Working Group: Volker Brendel, Ed Buckler, Karen Cone, Mike Freeling, Owen Hoekenga, Lukas Mueller, Marty Sachs, Pat Schnable, Tom Slezak, Anne Sylvester, and Doreen Ware. We thank the MaizeGDB Editorial Board for recommending, on a monthly basis, noteworthy primary literature. This Board currently includes: Tom Brutnell, Surinder Chopra, Karen McGinnis, Wojtek Pawlowski and Jianming Yu.

Submitted by:
Mary Schaeffer (Polacco)
Carolyn J. Lawrence

VI. MAIZE SEQUENCING STATUS REPORTS

THE MAIZE SEQUENCING PROJECTS
<http://www.maizegdb.org/sequencing_project.php>

This is a summary of Rick Wilson's talk at the 2006 Maize Meeting, posted at MaizeGDB and updated May 2006.

November 15, 2005, the NSF, USDA, and DOE announced their award of $32 million to the Genome Sequencing Center (Washington University;GSC), Cold Spring Harbor Laboratory(CSHL), the Arizona Genome Institute (AGI), Iowa State University (ISU), University of California-Berkeley, DOE Joint Genome Institute (JGI), University of Georgia and Stanford University for sequencing the maize genome. See also: <http://www.nsf.gov/news/news_summ.jsp?cntn_id=104608&org=BIO&from=news>

Project Descriptions
B73 A BAC by BAC approach
This effort - expected to require three years of work - will utilize a minimal tiling path of approximately 19,000 mapped BAC clones, and will focus on producing high-quality sequence coverage of all identifiable gene-containing regions of the maize genome. These regions will be ordered, oriented, and along with all of the intergenic sequences, anchored to the extant physical and genetic maps of the maize genome. Important features of the project include immediate release of preliminary and high-quality sequence assemblies, and the development of a genome browser that will facilitate user interaction with sequence and map data.

Mo17 Chromosome 10 by shotgun sequencing (JGI)
A whole genome shotgun (WGS) strategy is expected to capture ~90% of the maize genome. The WGS strategy is to be assessed using chromosome 10 of Mo17 flow sorted material as a test case.

B73 Project Input Data Descriptions
The Physical Map <http://www.genome.arizona.edu/fpc/maize/>

- Total Assembled Contigs: 721
  Equal to 2,150 Mb; 93.5% coverage of 2300 Mb genome
  Anchored: 421 ctgs; 86.1% the genome
  Average anchored contig size: 4.7 Mb
  Unanchored: 300 ctgs, 7.4% coverage
  Average unanchored contig size: 0.56 Mb
  189 of the 300 unanchored contigs are less than 10 clones
  Largest anchored contig 22.9Mb in Chr9
  Largest unanchored contig 6.7 Mb
- Total FPC Markers: 25,000
  STS markers: ~9,000
  Overgo Markers: 14,825
  Anchored markers: 1,918

The Tiling Path
Using the physical map, ~3,200 seed BACs are being chosen with an average spacing of 800 kb. These seeds are required to have:
1) at least one end sequenced,
2) both agarose and HICF fingerprints,
3) at least average insert size (~150 kb),
4) at least one overgo match.

Subsequently, BAC end sequences and fingerprint data are being used to extend the seed BACs into tiling path contigs for sequencing.

B73 Project Output Data Descriptions

- Sequence traces:
  Automatically deposited to the Trace Archive at NCBI within 24 hours of production (includes fosmid ends).
- BAC clone assemblies:
  Phase 1 HTGS_FULLTOP: 2 x 384 paired end attempts. Completed shotgun phase.
  Phase 1 HTGS_PREFIN Completed automated improvement phase.
  Phase 1 HTGS_ACTIVEFIN. Active work being done by a finisher.
  Phase 1 HTGS_IMPROVED. Finished sequence in gene regions. Improved regions will be indicated. Once order and orientation of improved segments are confirmed, a comment will be added to indicate this.

B73 Project Timeline

Year 1:
 Production sequencing for ~7,000 BAC clones (GSC).
 Sequence 0.55M (0.3X coverage) fosmid end pairs (GSC).
 Begin pre-finishing and finishing (GSC, AGI, CSHL).
 Finish ~4,500 BACs (GSC, AGI, CSHL)
 Begin genome assembly & annotation efforts (CSHL, ISU).

Year 2:
 Production sequencing for ~10,000 BAC clones (GSC).
 Finish ~10,000 BACs (GSC, AGI, CSHL).
 Continue genome assembly & annotation efforts (CSHL, ISU).

Year 3:
 Production sequencing for remaining BACs (GSC).
 Finish remaining (~4,500) BACs (GSC, AGI, CSHL).
 Continue genome assembly & annotation efforts (CSHL, ISU).

Accessing the B73 data:

- At NCBI http://www.ncbi.nlm.nih.gov/entrez/
  Use the nucleotide search :
  Zea mays[ORGN] AND HTG[KYWD] AND WUGSC[CTR]
  to pull the clone assemblies currently available.
- http://www.maizesequence.org (available by late summer 2006)

Genome assemblies:
 Annotated BAC clones assembled in the context of mapping and other data, displayed in Gramene. Dynamically updated as new data is available. No built-in delays; new builds, annotation and data will be made available as processing queues allow. See also p. 74, this volume.

Further information on the project can be accessed through the following links:
Maize Genome Sequencing Information Portal <http://www.maizegdb.org/genome/ >
(reviews of sequencing methods, the Request for Proposals, etc.)

The Genome Sequencing Center's Maize Page for B73
<http://genome.wustl.edu/genome.cgi?GENOME=Zea%20mays%20mays%20cv.%20B73&GROUP=7>

B73 and Mo17 FISH image shows repetitive sequences (including knobs; courtesy of Jim Birchler)
<http://www.maizegdb.org/genome/B73Mo17FISH.php>

MGSC: Gramene and MaizeGDB cooperate to provide access to sequences and related data

--Lawrence, CJ; Ware, D

The NSF, USDA, and DOE announced on November 15, 2005 that together they had funded the sequencing of the genome of inbred line B73 as well as chromosome ten of Mo17 (a project that aims simultaneously to evaluate shotgun sequencing strategies for large genomes and to investigate maize diversity). In addition, the USDA-ARS contributed the MaizeGDB project resources. Because Gramene will be the primary portal to the maize B73 sequences (which are to be annotated by the Ware group), a description of past and present interactions between MaizeGDB and Gramene is presented here. This contribution describes our groups' interactions and also explains current and planned access points and portals to the maize sequence data. For a description of the maize sequencing project's deliverables and timelines, see pp. 71-72 in this volume of the Maize Newsletter.

MaizeGDB and Gramene personnel began collaborating early on, and have been involved in developing shared resources like the Plant Ontologies, (http://www.plantontology.org) a set of terms that describe plant anatomy and developmental stages, for the last three years. This hierarchical vocabulary enables data to be integrated by the use of common terms across different databases to describe divergent datasets, such as EST collections, mutant strains, and stocks, so that they can be simultaneously searched and analyzed. This set of terms currently is in place at both MaizeGDB and Gramene, enabling the annotation of various data types at both repositories, and is a resource upon which many connections can be built (between MaizeGDB and Gramene, and also with other resources like TAIR, the Solanacea Genomics Network, the Virtual Plant Information Network, and other plant databases).

In addition to working together, members of the MaizeGDB and Gramene teams have been apprised of and involved in the development of both resources. For instance, Gramene PI L. Stein contributed to guiding MaizeGDB's development by serving on the MaizeDB to MaizeGDB Transition Steering Committee, and Gramene co-PI D.W. currently serves as a member of the MaizeGDB Working Group. Similarly, MaizeGDB director C.J.L. has participated in Gramene Scientific Advisory Board meetings during the past two years. Curators from Gramene attended the MaizeGDB curation tools workshop in Ames, Iowa in the fall of 2004, and a working meeting to integrate maps and molecular markers was co-organized by MaizeGDB and Gramene personnel and was conducted one evening at the 2005 Maize Genetics Conference. Ideas and data are exchanged between the two groups on a regular basis.

The first of a number of sequence data meetings between the Ware maize sequence analysis group and the MaizeGDB team is slated to take place in June of 2006 at the Cold Spring Harbor Laboratory. During this meeting, we will work to identify means to synchronize data release and make accessing maize sequence data easier for researchers, irrespective of data storage location. We also will explore methods for addressing feedback from maize geneticists that is relevant to both projects. We expect that a joint feedback mechanism may be in order, but the logistics and implementation of such a mechanism will require serious consideration and discussion. It is expected that outcomes from the June meeting will serve to guide both groups' development strategies to maximize accessibility to sequence data while minimizing duplication of effort.

At present, the Gramene and MaizeGDB websites are linked throughout by way of shared data, common nomenclature, and a standard set of linking rules. New linkages and entry points to data will be made available at both sites as they are identified. For a list of some existing linkages, see Tables 1 and 2. Datasets shared by both groups include sequences, BACs, loci, markers, maps, and ontology terms. These datasets will serve as the basis for creating new linkages to increase the interconnectedness of the two resources. We solicit ideas you might have for how to improve both MaizeGDB and Gramene. Please send all comments and suggestions to both MaizeGDB and Gramene by way of our groups' shared email address: feedback@maizesequence.org. Your help, guidance, and continued support are greatly appreciated!

Table 1. Links from MaizeGDB to Gramene that are already in place.

MaizeGDB Data Type	<Example Entry URL> and Link Placement to Gramene	Purpose
Sequences	<http://www.maizegdb.org/cgi-bin/displayseqrecord.cgi?id=AC149813> Right green bar, under "Search Tools".	Jump from MaizeGDB BAC data to the Gramene Finger Print Contig viewer
BACs	<http://www.maizegdb.org/cgi-bin/displaybacrecord.cgi?id=507533> Top of the page, in bold font.	Jump from MaizeGDB BAC data to the Gramene Finger Print Contig viewer
Loci	<http://www.maizegdb.org/cgi-bin/displaylocusrecord.cgi?id=12098> Right green bar, under "Search Tools".	View the locus within the context of its map location using CMap
Maps	<http://www.maizegdb.org/cgi-bin/displaymaprecord.cgi?id=143439> Right green bar, under "Other Map Views".	View the map visually using CMap

Table 2. Links from Gramene to MaizeGDB that are already in place.

Gramene Data Type	<Example Entry URL> and Link Placement to MaizeGDB	Purpose
BACs	< http://www.gramene.org/Zea_mays/cytoview?mapfrag=AC149813> Context menu for BAC on "Acc Clones" track.	Show associated marker data on MaizeGDB
Maps	<http://www.gramene.org/Zea_mays/cytoview?mapfrag=c0148C07> Context menu for clone on "FPC Map" track.	Show associated marker data on MaizeGDB
Markers	<http://www.gramene.org/Zea_mays/cytoview?contig=ctg129> Context menu for individual markers on "Markers" track.	Jump to marker info on MaizeGDB
Diversity	<http://www.gramene.org/db/cmap/feature?feature_acc=cmf1104a-ctg251-10> Cross-reference to MaizeGDB.	Jump to locus info on MaizeGDB

VII. BAC CONTIGS AND THEIR GENETIC ANCHORS

BAC contigs and CB positions of anchoring loci are from the maize FPC at Arizona (http://www.genome.arizona.edu/fpc/maize/). Genetic positions are based on the IBM2 high resolution map (http://www.maizegdb.org), computed from a 302 member panel of Stocks for inter-mated B73 x Mo17 recombinant inbreds. This mapping panel is available from the Maize Genetics Cooperation Stock Center and described by Lee et al. (Plant Mol. Biol. 48:453-461, 2003).

Columns:
Bins are provided for the locus that approximates, on this list, the boundary of a bin; when it corresponds to a Core Marker, which define the bin boundaries, the corresponding locus and coordinate information is in bold face.

Contig: the number of the contig, as defined in the current (July 2005) FPC build.

CB: the position in a contig for a marker based on the consensus band (restriction fragment) for clones. In maize the CB is 4900 bp (http://www.genome.arizona.edu/fpc/maize/).

IBM: the IBM2 cM position, for loci flagged by an asterisk (*). Other loci have the approximate value from the IBM2 2005 neighbors computation. Contigs anchored by unpublished loci are provided the genetic position listed in FPC for a contig; in many instances these anchorings are based on rice-maize synteny of markers. Most of the FPC markers, both SSRs and overgos, were developed from maize cDNAs (ESTs; Unigenes). (Gardiner et al., Plant Physiol. 134:1317:1326, 2004).

Locus: Loci flagged by an asterisk are taken from the IBM2 map, which uses the high resolution 302 member inter-mated IBM mapping population of Mike Lee. Other loci are from the IBM2 2006 Neighbors map, a serial projection of genetic maps stored in MaizeGDB onto the IBM2 frame.

Shaded areas on each chromosome are approximate centromere locations, based on the Genetiic 2005 map compilations of Ed Coe (MNL 79; MaizeGDB).

Submitted by
Mary Schaeffer (Polacco)

Chromosome 1

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
1.00	1	22	-0.12	bnlg149
		382	-0.01	umc1613
		413	0	umc1353
		416	0	umc1354*
		440	0	bnlg1124
1.01		454	2.5	tub1*
	2	115	10.5	dmt103b
		139	10.5	umc1177*
		159	16.5	umc1566*
		165	16.5	umc2183
	3	40	26.1	mmp102*
		40	31.1	AY110314*
		110	31.11	umc1619
		129	31.11	csu589
		229	48.7	lim179*
		330	48.71	AY107629
		343	52.59	csu680a
		343	48.71	umc1292
		344	48.71	bnlg1179
	4	45	68.3	mlo1*
		50	68.3	umc1041
		84	68.3	gpb1
		114	68.31	umc1106
		156	68.31	PCO072650
		222	82.8	bnlg1014*
		291	87.4	umc2012*
		372	88	umc1071*
		410	86.3	umc1269*
		412	88.3	umc1305
		422	88.3	smt2
		437	88.3	gst31
		483	89.2	umc1977*
		543	89.2	prc3
		551	91.5	umc1948*
	5	20	102.99	AY110853
		68	103	umc2215
		82	103	umc1685*
		382	108.3	umc1160*
		470	110.9	umc2224*
1.02		534	113.87	umc157a(chn)*
	6	171	124.69	bnlg1178
		172	124.69	esr2
		300	124.7	umc2225*
		325	133.6	umc1166*
		410	133.61	PCO128140
		488	141.8	umc1568*
	7	18	143.5	bnlg1429*
	8	48	160.53	pds1
		314	160.55	bnlg1627
		332	160.55	umc2191
		562	160.58	umc1467
		794	160.6	umc1976*
		819	165.8	umc2226*
		1033	166	mmp135*
	9	20	170	bnlg1953*
		371	170.04	umc1711
		621	170.06	bnlg1007
		846	114.7	gln6
		949	170.09	bnlg1803
1.03		1097	198.06	umc76a*
	10	98	204.95	umc2383

Chromosome 1

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		584	205	lim122*
	11	40	210.6	umc1403*
		392	210.64	umc2397
		405	219	bnlg1484*
		1167	225.4	AY109929*
		1230	225.41	umc2185
		1274	226.4	umc1397*
		1316	229.6	AY110052*
		1565	229.63	ibp2
		1739	229.64	PCO063726
		2076	257.4	umc1479*
		2156	257.41	csu145c(pck)
		2174	257.41	PCO074335
		2176	259.1	bnlg439*
		2177	259.3	bnlg1203*
		2425	260.7	fad8*
		2512	260.71	umc1701
		2587	260.72	pdc3
		2723	270.6	umc13*
		2758	278.1	AY110632*
		3042	278.13	rth3
		3153	278.14	p1
	12	12	287.14	p2
		97	287.14	AY110240
		396	287.18	AY107489
		576	287.19	umc1514
		632	302.8	AY110393*
		638	287.2	AW400087*
		680	287.2	bnlg182
		680	290.3	umc1880*
		680	290.1	bnlg1866*
		699	290.32	umc2171
		772	290.4	phi109275*
		825	290.41	asg45(ptk)
	13	120	292.4	lim432*
1.04	14	26	328.57	umc1452
		198	328.59	cdo38a(ntp)
		308	328.6	umc49c
		404	326.7	bnlg2238*
		494	326.71	bnlg1016
		494	320.9	mmp56*
		667	328.5	umc2124*
		685	328.71	umc1254
		723	328.71	les22
		1441	337.69	sod4*
		1446	340.7	csu737(npc)
		1636	337.71	umc2217
		1858	350.6	AY110330*
		2013	374.8	umc1917*
		2272	350.64	umc1144
	15		345	unpublished
	16	42	360.9	umc2227*
		324	360.93	umc2390
	17	237	386.4	bnlg1811*
	18	389	390.8	bnl9.11b(lts)*
		472	391.8	umc2228*
	19	552	392.73	aoc1
		844	392.75	umc1770
	20	125	398.2	bnlg2295*
		156	397.3	umc2229*
1.05	21	191	405	csu3*

Chromosome 1				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		309	408.21	*csu694b(uce)*
		367	405.02	*umc1243*
		367	401.3	*umc2112**
		392	401.2	*bnlg2086**
		673	413.06	*mtl2*
	22		406	unpublished
	23	634	410.06	*bnlg1884b*
	24		415	unpublished
	25	15	422.68	*umc1734*
	26	177	434.61	*umc1297*
		498	430.6	*umc1515**
		599	430.62	*myc7**
	27	352	432.4	*umc2230**
		588	433.6	*AY111680**
	28	494	437.3	*AY109678**
	29	611	438.8	*umc1461**
		1029	439	*csu1138**
		1187	439.01	*umc1689*
	30	1899	440	*umc1076**
		2729	473.5	*obf1*
		2852	441.2	*mmp101**
		2883	445.5	*AY110396**
	31	71	450.8	*umc1676**
		828	450.87	*umc1611*
		1403	450.92	*AY106439*
	32	234	453.5	*umc2231**
		447	453.52	*umc1626*
		700	453.54	*bsd2*
	33	22	463.9	*CL14065_1*
		210	467	*umc1906**
		321	467.9	*umc1903**
	34	23	464.7	*AI855190**
		97	471.7	*umc1395**
		217	473.2	*umc1321**
		276	475.9	*umc1603**
		276	474.5	*umc2233**
	35		476	unpublished
	36	116	485.84	*umc1323*
		745	485.9	*uaz276**
		1138	485.94	*mbd106*
1.06		1536	499.22	*gpm4*
	37	339	508.18	*umc2083*
		435	508.19	*uce1*
		556	508.2	*umc1812**
		557	508.2	*umc1754*
		837	508.23	*csu61b*
		992	508.24	*csu92a*
		1001	508.24	*umc1508*
		1013	517	*umc1590**
	38	68	527.6	*bnlg2057**
		590	529	*umc2234**
		726	535.1	*umc1123**
		726	532.8	*bnlg1598**
		991	541.3	*AY104360**
		1038	541.3	*umc1281*
		1038	541.3	*umc1398*
		1047	544.2	*mmp156**
		1277	548.3	*bnlg1057**
	39	70	548.4	*umc1396**
		133	550	*umc2235**
		204	555.71	*umc1919**

Chromosome 1				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
	40	82	557.6	*bnlg1615**
		157	558.5	*csu805**
	41	19	570.8	*ntf1**
		41	570.8	*umc1668*
		146	587	*umc1035**
		148	583.3	*mmp123**
		174	587	*PCO116807*
		201	588.2	*umc1709**
		490	593.8	*umc1924**
		522	593.8	*umc1335*
		731	604.8	*umc2236**
		855	606.5	*umc1925**
1.07		**859**	**607.3**	***asg62****
		1039	607.32	*hm1*
		1236	618.5	*umc2237**
		1414	618.52	*umc1122*
		1527	630.6	*umc2239**
		1535	642.27	*umc2396*
	42	27	648.5	*umc1661*
	43	218	649.5	*bcd98a**
		373	652.08	*umc1356*
		373	652.08	*umc1374*
		452	653.4	*umc1358**
		586	656.7	*AY111834**
	44	76	658.6	*bnlg1556**
		555	658.65	*umc1833*
		588	658.65	*umc1486*
		727	658.67	*umc1706*
		1505	658.74	*umc1278*
		2620	658.85	*umc2064*
		2841	658.88	*bnl17.15b(bt2)*
		2846	658.88	*btl2*
	45	175	693.6	*mmp173**
		218	693.6	*umc2387*
	46	21	700.5	*bnlg1025**
		103	706.4	*AY110356**
		131	711.5	*umc1128**
		310	714.4	*umc1147**
		593	735.2	*AY110313**
		658	737.24	*umc1848*
		758	740.4	*AY110191**
1.08		**938**	**722.4**	***umc128****
		1009	722.3	*AY110159**
		1266	721.9	*umc1245**
		1274	741.51	*umc59c*
		1619	744.7	*cdo98b**
		1737	747.9	*umc1998**
		1766	747.9	*bnlg1629*
	47	151	755.2	*bnlg2228**
		152	756.5	*umc83a**
		244	756.51	*umc2029*
	48	49	760.3	*lim254**
		244	770.4	*umc1955**
		285	770.4	*umc1085*
		586	774.5	*umc2181**
	49	389	776.51	*mmc0041*
		452	776.51	*umc1838**
		621	781.6	*umc1446**
		717	785.3	*an1**
		728	784.7	*mmp22**
		759	785.3	*bz2*

Chromosome 1

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		943	793.05	*AY110349**
	50	98	800.7	*umc1991**
		265	800.72	*bnlg1643*
		619	805.3	*umc1383**
		693	806.5	*umc2240**
		719	806.5	*umc2385*
		720	806.5	*umc1843*
	51	145	807.57	*AY106137*
	52	262	809.86	*nfc103a*
		453	809.88	*umc2116*
		495	809.89	*vp14*
		623	809.9	*umc1914**
		631	811	*AY109506**
1.09		**659**	**812.3**	***cdj2****
	53	63	839.29	*glb1**
	54	356	847	*umc1298*
		357	847	*bnlg1331**
	55	56	842.3	*umc2047**
		410	825.14	*umc27b*
	56	418	849.01	*lpe1*
	57	11	883.03	*AY112283*
		46	883.03	*ole4*
		125	883.04	*umc2028*
		295	883.06	*umc1306*
		402	883.07	*bnlg1720*
		410	883.07	*tb1**
		693	886.1	*umc1431**
1.10		**833**	**886.9**	***umc107a(croc)****
		834	886.9	*gln2*
		1035	889.9	*AI665421**
		1296	889.93	*PCO087393*
		1403	889.94	*umc1290*
		1436	890.9	*AY110019**
		1520	890.91	*kn1*
		1579	890.91	*knox3*
		1880	898.7	*umc2149**
		1887	898.7	*bnlg1268*
		1939	927.67	*AY104234*
		2048	901.85	*adh1**
		2289	907.1	*BE639426**
		2322	907.11	*umc1885*
		2355	907.12	*umc1534*
		2355	913.4	*bnlg1671**
		2614	934.5	*uaz130a(tlk)**
		2838	926.3	*PCO095183*
	58	30	927.4	*mta1*
		128	927.4	*umc1774*
		209	927.4	*phi308707**
		271	927.4	*umc2223*
	59		930	unpublished*d*
	60		951.2	unpublished
1.11	**61**	**291**	**963.6**	***umc161a****
	62	410	972.99	*umc1500*
		484	973.97	*csu63a(cdj)*
		488	973	*phi265454**
		675	1,007.60	*umc1553**
		707	987.3	*AY110426**
	63	50	1,014.90	*umc1681**
		60	1,014.90	*ohp1*
		108	1,019.10	*umc1129**
	64	36	1,030.98	*csu604a(trh)*
		152	1,030.99	*umc1862*
		184	1,030.99	*bnlg2331*
		255	1,031.80	*umc2242**
		265	1,031.00	*umc2241**
		272	1,031.80	*csu33b*
		280	1,031.80	*ccr1*
		282	1,031.80	*umc1737*
		290	1,031.80	*bnlg1055*
		318	1,034.30	*umc1118**
		338	1,034.30	*cesa5*
		492	1,034.32	*bnlg667a*
		625	1,041.78	*csu134a(thf)*
		642	1,034.33	*AY109096*
		773	1,051.10	*umc1744**
		815	1,051.10	*ids1*
		832	1,051.11	*chi1*
		860	1,051.11	*umc1220*
		861	1,054.20	*umc84a**
		965	1,054.21	*AY106825*
		971	1,054.21	*bnlg2123*
		972	1,054.21	*bnlg131*
		972	1,055.90	*umc1630**
		986	1,055.90	*AY109128*
	65	32	1,096.47	*bnlg504*
		55	1,096.47	*AY112175*
		249	1,096.49	*CL62610_1*
		262	1,099.30	*umc2045**
		307	1,098.40	*fdx3**
		311	1,098.40	*umc2243**
		333	1,096.50	*umc1725*
		335	1,096.50	*umc1331**
		339	1,097.40	*phi227562**
		423	1,103.00	*phi064**
1.12	**66**	58	1,121.90	*tufm1**
		98	1,119.20	*umc1819**
		120	1,117.10	*umc1605**
		120	1,120.30	*umc2244**
		185	1,122.90	*AY109916**
	67	30	1136.0	*umc1499*
		36	1,136.00	*csu1114*
		229	1,136.02	*umc1797*
		290	1,136.03	*AY104686*

Chromosome 2				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
2.01	**69**	60	47.4	*umc1165**
		81	47.4	*umc2363*
		109	47.4	*csu642*
		133	47.41	*bnlg1338*
2.02		180	57.6	*umc1542**
		196	57.6	*umc1227*
		374	77.7	*umc1265**
		591	87.8	*BE640649**
		694	87.81	*umc2403*
		698	87.81	*sgb101*
	70	78	92.6	*umc1824a**
		199	92.8	*umc1823**
		199	92	*umc1961**
		250	93.3	*mmc0111**
		260	93.3	*bnl7.49c(hmd)*
		359	94.4	*AY109516**
		397	94.4	*CL52019_1*
		629	94.43	*bnlg1302*
		676	94.43	*dmt102b*
		720	94.44	*umc1934*
		728	122.4	*eks1**
	71	305	148.1	*bnlg1327**
		461	154.6	*umc1261**
		461	152.83	*umc1262**
		468	154.71	*bnlg125*
		468	154.71	*csu1091*
		468	154.71	*csu1113*
		522	156.6	*umc1422**
		533	156.6	*gpm7*
	72	103	156.56	*umc2193*
		127	163.5	*AY106040**
2.03		**161**	**164.55**	***umc6a****
	73	82	164.54	*si605074C02*
	74	19	182.3	*umc44b**
		124	182.31	*nfd102*
		245	182.32	*bcd855a(ext)*
		351	197.15	*b1**
		502	197.17	*umc1845*
		545	203.1	*mmp33**
		835	217.32	*ole1**
		938	217.33	*sdg104*
		1081	217.34	*AY107034*
		1111	221.4	*AI920398**
		1259	221.41	*bnlg2248*
		1362	221.42	*umc1769*
		1619	221.45	*umc1555*
		2020	227.1	*bnlg1064**
2.04		**2255**	**243.3**	***umc34****
		2255	244	*phi109642**
		2305	236.4	*AY104214**
		2337	244.7	*bnlg381**
		2337	244.01	*si606023F08*
	75	15	250.1	*umc1026*
		15	250.1	*umc1024**
	76	9	251.1	*umc2247**
		304	251.13	*AY103944*
		499	267.8	*umc2248**
		589	262.6	*AY110266**
		600	266.8	*umc1326**
		611	268.4	*umc259b**
		770	269.6	*umc1448**

Chromosome 2				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		805	269.6	*cta1*
	77	27	273.61	*PCO140184*
		604	273.67	*hag103a*
		851	273.69	*CL58207_1*
		918	273.7	*umc1465**
		1019	274.9	*umc1541**
		1402	284.7	*prp2**
		1408	284.7	*CL10221_1*
		1450	284.7	*umc2032*
	78	76	293.31	*grf1**
		118	293.32	*umc1579*
		118	294.2	*umc1580**
		171	293.8	*lim86**
		171	294.2	*bnlg1018**
		227	294.21	*cdo1328b*
		278	295.1	*bnlg1175**
		444	295.8	*umc2251**
		528	296.3	*umc2249**
		797	292.87	*csu56c(ohp)*
		1219	296.37	*AI714808*
		1294	306.3	*bnlg108**
		1327	306.3	*bnlg1909*
		1507	310.2	*umc1259**
	79	189	313.5	*umc2030**
		315	314.4	*umc1861**
		497	314.41	*umc1285*
		1038	316.7	*umc2088**
		1191	316.72	*umc1485*
		1194	323.3	*hrg1**
		1590	320.7	*umc2079**
	80	120	328.93	*hda102*
		398	328.96	*AY112119*
		825	329	*umc2250**
	81	53	332.17	*bnlg1613*
		339	332.2	*umc2125**
	82	400	339.3	*umc1454**
		402	339.3	*umc1455*
		615	339.32	*umc1410*
	83	172	341.32	*umc1007*
2.05	**84**	565	342.38	*npi220d*
	85	135	344.8	*umc1635**
	86	408	344.4	*umc1581**
		2332	345.72	*zpu1**
		2775	345.74	*cdo456b*
		2830	345.74	*umc1884*
		2830	349	*AY109687**
	87	381	346.47	*AY107012*
		1118	346.5	*mmp119**
	88	59	346.46	*bnlg1887*
	89	1973	352.21	*hsbp1*
		3713	352.4	*AW681281**
	90	366	357.45	*umc1459*
		394	358.6	*mmc0401**
		726	359.1	*umc2252**
		893	359.12	*AY111877*
		986	357.5	*umc2110**
		1192	361.2	*umc1028**
	91	112	369.3	*umc1079**
		273	368.8	*bnlg1831**
		368	364.5	*AY110336**
2.06		**371**	**369.32**	***umc255a***

Chromosome 2				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		565	369.34	*pbf1**
		619	369.34	*bnlg371*
		1141	369.38	*umc1235*
		1210	373.5	*bnlg1036**
		1653	373.57	*umc1156*
		1966	373.62	*csu747a(arf)*
		2653	375.3	*umc1658**
		3554	376.1	*umc2253**
		3899	376.14	*bnlg1396*
		3992	377.4	*umc2178**
		4070	377.41	*umc2194*
		4200	377.42	*umc2192*
	92	52	378.7	*umc2254**
		156	379.2	*bnlg1138**
		287	379.28	*akh2*
		498	379.4	*umc1923**
		608	380	*umc1080**
		626	380.6	*umc1755**
	93		380.2	unpublished
	94	81	380.8	*AY109981**
	95	135	381.8	*umc1004**
	96	56	382.8	*PCO063114*
		781	382.87	*umc1749*
		846	382.88	*umc2023*
	97	17	394.89	*umc2019*
		48	394.89	*umc1946*
	98	45	401.94	*umc2402*
		166	401.95	*umc1637*
		190	401.95	*bnlg1329*
		382	401.97	*amy3*
		463	401.98	*AI861369*
		742	414.1	*umc2129**
		789	414.1	*umc1497*
		1122	414.14	*umc2380*
	99		416	unpublished
	100	10	422.68	*mmc0271*
		10	178.78	*mmc0231**
		163	427.9	*AY110410**
		187	422.7	*umc1890**
	101	354	433.4	*umc2205*
	102		450	*unpublshed*
	103	62	452.19	*bnlg1633*
		100	453.8	*phi251315**
		155	452.2	*AY109917**
		245	453.81	*bnlg1267*
		555	453.85	*bnlg198*
		555	453.85	*bnlg1335*
		868	475.1	*umc1560**
		877	474.8	*bnlg2077**
		925	475.11	*umc1554*
		980	475.11	*umc4a*
		1136	475.12	*umc1536*
2.08		**1234**	**478.7**	***asg20****
		1283	480.7	*umc1049**
		1296	482.2	*mmp116**
	104	79	509.12	*bnlg1662*
		143	509.13	*umc1126*
		154	509.13	*bnlg1721*
		192	509.13	*bnlg1767*
		501	509.16	*bnlg1169*
		687	509.18	*umc1526*

Chromosome 2				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		837	509.2	*umc2005*
		869	509.2	*bnlg1233**
		895	515.85	*hda109*
		927	515.8	*AI668346**
		1053	520.5	*phi435417**
		1167	522.4	*umc1947**
		1252	136.1	*umc1604**
		1437	529.2	*bnlg1316**
		1739	529.23	*chc101b*
		1739	538.8	*AY109645**
		1893	538.82	*PCO102097*
		1898	538.82	*umc1992*
		1898	544.4	*umc2085**
		1954	547.68	*npi298a**
		1959	548.5	*umc1633**
		2075	562.5	*mmp34**
	105	92	572.4	*bnlg1606*
		92	573.3	*cdo38c(ntp)**
		98	572.4	*mmc0381**
		99	573.3	*bnlg1141*
		153	573.6	*bnlg1746**
		183	573.6	*umc1798*
		496	577.6	*bnlg1940**
	106	161	579.81	*umc2202*
	107	9	584.3	*umc1516**
		19	584.3	*gpm16*
	108	15	591.47	*umc2374*
		225	591.49	*umc1230*
2.09		**383**	**591.5**	***umc49a****
		474	591.51	*umc1551*
		598	591.52	*bnlg1520*
		778	343.7	*umc1252**
		778	600.7	*umc1256**
		891	601.6	*AY109592**
		1172	601.63	*umc1525*
		2027	601.71	*umc1736*
	109	279	650.1	*bnlg469b**
		317	654.8	*bnlg1893**
		334	654.8	*csu109a*
		637	692.4	*umc2184**
		670	692.4	*umc2077*
		672	681.8	*AY110389**
		707	694.6	*mmp183**
	110	46	713.1	*AY109586**
		120	712.1	*phi101049**
		186	713.11	*umc2214*
		331	725.3	*AY111236**
		398	725.31	*AY106674*

Chromosome 3				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
3.00	**111**	265	0	*umc2118**
		296	5.59	*g2**
3.01		334	5.6	*umc1931**
		334	9.5	*umc2255**
		338	7.5	*phi453121**
		340	7.1	*umc1746**
		352	9.5	*umc32a*
		420	9.5	*AY106313*
		427	9.5	*umc1793*
		427	11.2	*umc1780**
		427	11	*phi404206**
		491	21.8	*umc1394**
		520	23.4	*umc2256**
		539	28.2	*umc1970**
		543	29.2	*umc2071**
		630	30.5	*umc2257**
		673	30.5	*AY112199*
		679	35.5	*umc1892**
		687	38	*phi104127**
		699	38.7	*umc2049**
		704	38.7	*umc2377*
		709	37.35	*umc2376*
		712	38.7	*cdo345b*
3.02		**766**	**60**	***csu32a****
		916	67.2	*umc1458**
		1029	77	*bnlg1144**
		1054	78.5	*umc1886**
		1063	78.5	*bnlg1325*
		1069	78.5	*csu230*
		1102	78.51	*bnlg1523*
		1127	78.51	*umc1814*
		1351	78.53	*me3*
		1400	95.4	*AY109549**
		1443	98.42	*cko1**
	112	26	103.3	*bnlg1647**
3.03		**87**	**109**	***asg24a(gts)****
		353	127.8	*umc2369*
		353	127.8	*umc2258**
		359	131.7	*umc2259**
		436	129.4	*bnlg1447**
		447	132.59	*bnlg1904*
	113		131	unpublished
3.04	**114**	**20**	**152.7**	***asg48****
		137	153.75	*csu242*
		297	157.3	*uaz159b**
		375	159	*umc59e**
		375	159	*umc1030**
		448	163.5	*umc1772**
		452	163.5	*umc1729*
		685	165	*umc1425**
		819	166.9	*umc2000**
		972	168.01	*tpi4*
		980	168.02	*bnlg2136*
	114	1005	168.02	*umc1965*
	115	180	177.4	*umc1495**
		181	176.6	*umc2158**
		194	177.4	*umc1351*
		543	181.7	*umc2033**
		605	181.1	*umc1392**
	116	45	189	*umc1742**
		258	189.03	*rz543a*

Chromosome 3				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		481	190.2	*umc2117**
		548	191.1	*umc1655**
		548	190.6	*bnlg1019a**
		559	190.8	*bnlg1452**
	117	24	191.97	*ocl1*
		152	191.98	*umc1087*
		152	190.8	*bnlg1113**
		179	190.8	*umc1717**
		792	192.04	*bnlg2047*
		956	192.05	*umc2260*
		1017	192.06	*AY107193*
		1287	208.6	*mmc0132**
		1476		*umc1810*
		1687	210.4	*umc2261**
		2111	212.7	*mmc0312**
		2111	213.6	*umc1908**
		2390	213.63	*PCO107756*
		2476	214.7	*umc2262**
		3684	214.82	*npi220b*
		3737	214.83	*umc1347*
	118	489	227.8	*umc2263**
		681	337.23	*te1*
		1812	318.2	*umc2020**
	119	297	228.2	*PCO068796*
		362	228.2	*umc1504**
		869	228.5	*mmp29**
	120	244	238.1	*AY110403**
		636	244.7	*AY110297**
		661	266	*umc1683**
		694	266	*PCO141323*
		2956	266.03	*bnlg1957*
	121	975	254.6	*AY110151**
		1391	254.64	*AY111333*
		2034	261.1	*umc2264**
		2300	261.11	*bnlg602*
		2766	261.12	*umc1750*
		2776	262.9	*mmp9**
		2782	269.4	*umc1449**
	122	70	283.89	*cdo1160b(kri)*
	123	231	279.3	*umc1527**
		432	279.32	*umc1616*
		525	279.33	*bnlg1628*
		543	280.4	*umc1773**
		1344	290.6	*umc2002**
		1489	290.62	*gpm14*
3.05	**124**	**268**	**297.9**	***umc102a****
		337	299.2	*umc1174**
		337	301	*umc1600**
		704	301.03	*AI714716*
		876	301.04	*umc1300*
		1514	306.06	*phys2**
		1870	306.09	*abp1*
		1934	306.1	*bnlg1601**
	125	89	312.77	*chr109b*
		310	312.8	*umc1102**
		310	313.4	*bnlg1035**
		408	315.4	*AY110352**
		595	315.42	*csu439(trm)*
	126	43	318.14	*atp1*
		398	319.2	*umc1167**
		680	318.2	*umc2020**

Chromosome 3				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		696	318.2	*mmc0022**
		696	318.2	*umc2020**
		1099	319.26	*umc2155*
		1116	319.26	*umc2127*
	127	54	326.2	*AY112215**
		514	326.25	*csu706*
	128	305	331.3	*AY111507**
		476	334.6	*AY111541**
		1033	334.66	*bnlg1117*
	129	491	343.78	*ldp1*
	130	73	345.77	*umc1954*
	131	142	346.8	*myb2**
		388	346.83	*cdo109*
		411	346.83	*umc1839*
		440	346.83	*si618046E03*
		745	354	*umc2265**
		821	361.1	*csu636**
		882	358.3	*sps2**
	132	876	371.4	*umc1973**
		947	384.9	*AY111296**
		1142	384.92	*umc1400*
		1168	384.92	*umc2166*
		1192	388.1	*AI770873**
3.06		**1317**	**391.4**	***bnl5.37a****
		1339	390.3	*umc1539**
		1499	391.41	*umc1593b*
		1702	394.8	*umc1311**
		1723	394.8	*bnlg1449*
		1963	398.4	*umc1730**
		2096	401.2	*umc1027**
	133	141	402.94	*npi268b*
	134	85	411.6	*bnlg1063a*
		128	411.6	*umc1266**
		240	411.61	*npi432*
		277	411.62	*bnlg1798*
	135	171	434.3	*umc2266**
		370	434.33	*bnlg1047a*
	136		445	unpublished
	137	197	452.7	*umc60**
		213	452.7	*umc2408*
		236	452.7	*umc1951*
	138	84	461.1	*umc2268**
		613	461.15	*csu351*
		630	461.15	*umc2076*
		700	482.3	*umc2269**
		716	481.6	*bnlg1951**
		1002	482.33	*umc1985*
		1037	482.33	*bnlg1931*
		1049	488	*csu191**
		1245	491.4	*bnlg1160**
		1306	491.41	*csu264*
		1306	491.41	*umc2381*
		1338	491.41	*csu180*
		1338	491.41	*umc2169*
		1437	494	*umc2270**
		1438	494	*umc2271**
		1682	494.02	*CL13054_1*
		1732	503	*lim424**
		1880	507.2	*AY111125**
	139	177	511.5	*dupssr17*
		177	511.5	*bnlg197**

Chromosome 3				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		542	512.7	*AI770795**
		614	512.71	*si618016E09*
3.07	**140**	348	540.2	*umc1135**
		348	538.2	*umc2050**
	141	25	544.18	*umc1690*
		101	544.19	*si605077F08*
		228	544.2	*umc2272**
		383	544.4	*umc1528**
		452	544.6	*umc1399**
		459	552.67	*csu567(ces)*
		501	544.6	*bnlg1605**
		775	544.63	*umc1659*
	142	297	562.1	*AY104511**
		371	567.6	*umc1489**
		380	568	*umc2273**
		417	568.3	*umc1404**
		457	578.05	*cdo1160c(kri)*
	143	84	570.14	*umc1286*
		291	575.18	*nfc101*
	144	43	579.49	*AY106518*
		129	579.5	*umc1825**
3.08	**145**	28	597.53	*AW258116*
		741	597.6	*AY105849**
		766	597.6	*gpm3*
		813	597.61	*bnlg1779*
		900	609.2	*umc1140**
		913	610.2	*umc2275**
	146	124	617.5	*umc1915**
		137	618.6	*bnlg1108**
		591	618.65	*sdg115*
		682	633.8	*umc1320**
		688	638.3	*AY109934**
		689	652.4	*umc2276**
		718	634.8	*umc1273**
		763	652.41	*si946021A07*
	147	195	653.81	*csu397(cah)*
3.09	**148**	77	699.2	*csu303**
		112	699.2	*sh2*
		189	702.2	*csu845**
	149	50	702.19	*AY111254*
		196	702.2	*bnlg1257*
		264	702.21	*bnlg1182*
		506	738.7	*umc2152**
		570	747	*umc2008**
		672	748.5	*umc1813**
		710	747.5	*umc2277**
		711	747.5	*cdo665a*
	150	487	759.89	*mmc0001*
		496	759.89	*csu58a*
		567	759.9	*npi425a**
		589	760.9	*bnlg1496**
		769	769	*AY110567**
	151	135	806.9	*umc1361*
		139	806.9	*umc1052*
		184	806.9	*umc1641**
		617	806.94	*nph1*
		721	806.95	*umc255b*
		833	806.97	*umc2048*
		895	826.71	*umc1136*
		895	806.97	*umc1639*
	152	62	826.9	*bnlg1098*

Chromosome 3

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		290	826.93	*csu728a*
		307	826.93	*plt2**
	153	14	828.9	*umc1594**
		60	828.9	*cyp1*

Chromosome 4				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
4.01	**155**	93	15.8	*bnlg1370*
		147	15.8	*msf1**
		211	23.4	*umc1228**
		216	23.4	*umc2148*
		220	22.9	*umc2279**
		240	24.6	*umc123**
		312	24.61	*cyp3*
		315	37.5	*bx4**
		329	37.5	*umc1276*
		385	46.6	*cyp5**
	156	138	81	*bnlg1318*
		161	81	*umc1759**
		165	81	*umc1758*
		165	81	*umc1757**
4.02		**165**	**81**	***phi295450****
		188	71.87	*umc2409*
		213	81	*umc2150*
		248	101.1	*umc1943**
		251	101.1	*umc1509*
		445	101.12	*PCO146629*
	157	616	116.15	*umc1288*
		616	116.15	*umc1294*
4.03	**158**	**19**	**135.1**	***umc31a****
		19	135.7	*AY110398**
		123	141.6	*umc2082**
		330	143.4	*csu235**
		411	147.21	*adh2**
	159	157	152.9	*AY110253**
		240	152.91	*pdi1*
	160	246	158.8	*umc2280**
		246	158.6	*umc2281**
		341	181.4	*umc1902**
		458	196.4	*umc2039**
		489	196.4	*umc2211*
		539	200.3	*pgd3**
		539	214.53	*umc1821*
		944	205	*wip2**
4.04		**1132**	**210.83**	***npi386a(eks)****
		1186	210.83	*umc49d*
	161		213	unpublished
	162	182	218.48	*uaz246a(mbf)*
		343	218.5	*umc1117**
		513	223.6	*lim415**
		560	228.4	*umc1652**
		740	228.42	*psb3*
	163	328	232.13	*ocl5a*
		511	232.15	*zp1**
		511	736.7	*cat3**
		804	237.8	*bnlg490**
4.05	**164**	143	250.75	*bm3*
		240	250.76	*umc2206*
		609	250.8	*umc2061**
		757	250.82	*umc1662*
		766	250.82	*AY107128*
		968	254.9	*umc2282**
		1109	252.15	*gpc1**
		1141	253.9	*csu474(rpS14)*
		1428	253.93	*bnlg1937*
		1859	253.97	*umc1382*
		2236	254.01	*bnlg1217*
		2266	271.4	*umc1964**

Chromosome 4				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		2272	270.3	*umc1303**
		2449	268.4	*bnlg1265**
		2521	698.9	*umc1180**
		2774	271.41	*bnlg252*
		2881	271.41	*umc1390*
		3322	274.7	*AY110290**
	165	271	279.79	*chr112a*
		1051	279.87	*csu661*
		1283	279.9	*csu509**
	166	745	287.3	*umc1175**
		746	287.3	*akh1*
	167	278	289.26	*bnlg1729*
	168	61	295.2	*umc1511**
	169	117	298.1	*umc1791**
		481	298.11	*umc1851*
	170		298.5	unpublished
	171	966	299.9	*bnlg1755**
		1182	298.9	*mmp140**
	172	498	294.3	*umc1953**
		503	294.4	*umc2283**
		1051	294.45	*umc1451*
		1090	294.45	*umc1548*
		1209	294.46	*umc1317*
		1371	294.47	*umc1896*
		1676	294.5	*umc1895*
		1704	294.5	*pep7*
	173		300	unpublished
	174	149	300.72	*umc1362*
		161	300.72	*umc2054*
	175		301	unpublished
	176	69	302.49	*csu34b(rpS8)*
		230	302.5	*umc1142**
	177	137	303.4	*csu39*
	178	299	304.31	*CL65845_1*
	179	112	305.2	*umc1702**
		456	326.5	*AY110355**
		611	320.4	*AY110562**
4.06		1110	331.3	*mmc0371**
		1228	333.2	*umc1945**
		1248	332.4	*umc2284**
	180		335	unpublished
	181	441	362.34	*umc2391*
		934	362.39	*bnlg1621a*
		1060	362.4	*AY110310**
		1454	362.44	*umc1299*
	182	173	373.3	*rz567b(klc)**
		293	373.31	*umc2070*
		372	373.32	*umc1869*
		1234	373.4	*umc1329*
		2046	392.2	*bnlg2291**
		2082	392.4	*bnlg1137**
		2331	392.42	*bnlg1784*
4.07		2756	411.3	*umc2038**
		2843	411.31	*umc1651*
		2859	414.2	*umc19**
		3174	414.23	*umc1994*
		3220	414.24	*umc1620*
		3222	414.24	*umc1847*
		3545	428	*bnlg1189**
		3791	428.02	*PCO119336*
		3854	428.03	*csu672b*

Chromosome 4				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
4.08		4044	443.2	*umc1775**
		4071	443.2	*uaz171*
		4185	443.21	*mmc0341*
		4186	443.21	*umc2009*
		4359	452.1	*AY109534**
		4381	455.9	*mmp3**
		4466	455.91	*umc1043*
		4466	455.91	*umc1871*
	183	321	462.1	*umc1476**
	184	16	463.3	*gol1**
		202	463.32	*pdh1*
		434	463.34	*bnl8.45b*
		445	463.34	*umc2404*
		580	467.1	*bnlg2244**
		583	467.1	*umc2384*
		632	467.1	*bnlg1927*
		655	467.11	*umc1418*
		696	470.6	*umc1899**
		741	470.6	*PCO129009*
		766	475.7	*bnlg2162**
		844	475.71	*umc2365*
		905	475.71	*umc2041*
		946	475.72	*zrp4*
	185	19	514.89	*umc1612*
		138	514.9	*umc2285**
	186	33	515.91	*umc1086*
	187	344	522.06	*ssu1**
	188	12	532.23	*mmc0321*
		134	532.24	*cdo365(pet)*
		136	532.24	*csu166a*
		307	532.26	*umc1051*
		400	531.7	*umc2187**
		636	532.29	*CL12681_1*
		1039	536.9	*AY110989**
		1043	536.9	*PCO136722*
		1074	535.4	*AY109980**
		1115	535.4	*umc1371*
		1379	536.3	*AY105971**
		1428	535.5	*umc1856*
		1494	535.5	*umc1132**
		1634	535.5	*umc2153*
	189	118	562.75	*umc1313*
	190		536.7	unpublished
	191	177	553.7	*umc2286**
		188	554.1	*umc2188**
4.09	**192**	142	561.46	*umc1559*
	193	396	561.5	*AY110170**
		495	559	*umc52**
		744	561.52	*umc1039*
	194	49	565.38	*umc1834*
		123	565.39	*csu704*
		307	565.4	*lim446**
		437	565.41	*umc2200*
	195	241	574.8	*umc2139**
	196	292	574.81	*ensl002b*
		497	574.83	*PCO104784*
		646	574.84	*umc1939*
		646	581.8	*umc1940**
		652	581.8	*AY111962*
	197		612	unpublished
	198	21	616.7	*AY110064**

Chromosome 4				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		31	619.4	*umc2287**
		32	618.1	*umc1328**
	199	345	601.34	*bnlg1023a*
		845	601.39	*AY107200*
		940	601.4	*rz599b**
		1141	601.42	*umc1631*
		1150	601.42	*rpd3*
		1175	601.42	*umc1820*
	200	19	635.16	*umc1740*
		224	635.18	*umc2360*
		376	635.19	*umc2138*
		386	635.2	*umc2137*
		388	635.2	*umc2382*
		430	635.2	*AY110231**
		587	635.21	*sbp2**
	201	83	657	*umc2046**
		140	657.01	*PCO088312*
		153	655	*umc1101**
		207	657.01	*AY107910*
4.10		**283**	**669.8**	***php20608a****
		288	670.2	*bnlg589**
		301	670.2	*csu758*
		314	670.2	*umc1503*
		493	670.22	*umc1720*
		596	670.23	*umc1699*
		610	687.8	*umc1109**
		641	687.8	*umc2044*
		641	692.1	*umc2288**
	202	84	707.79	*PCO109372*
		139	707.8	*umc2289**
		139	715.5	*AY109611**
		164	708.5	*AY109859**
		165	716.21	*bnlg1337*
		182	716.21	*AY111822*
		198	716.21	*umc1719*
		198	728.5	*umc169**
		208	730.34	*wee1*
		296	730.34	*umc1716*
		323	736.7	*bip2**
		327	736.7	*cat3**
		372	740.7	*mmp182**
		372	739.3	*umc1649**
	203	55	748.3	*umc1707**

Chromosome 5				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
5.01	**205**	49	115.56	*bnlg1836a*
		82	115.56	*bnlg143*
	206	13	124.69	*umc1766*
		99	124.69	*umc1365*
		183	124.7	*umc1781*
		227	124.7	*umc2036**
5.02	**207**	**13**	**147.5**	***umc90***
		71	147.6	*umc66c(lcr)*
		100	150.9	*bnlg565**
	208	80	147.5	*tua4**
		218	156.9	*umc1587**
		363	160.2	*umc107b(croc)**
		364	160.2	*umc1894*
		453	160.21	*cdo542*
	209	128	189.77	*ole3*
		142	189.77	*umc2115*
		261	189.78	*bnlg1660*
		375	189.79	*AY110835*
		396	189.79	*bnlg105*
		435	189.8	*umc1761*
		466	189.8	*bnlg1879**
	210	54	196.89	*umc2167*
		132	196.9	*umc2293**
		160	196.9	*tbp2*
		231	196.91	*rps15*
5.03		347	203.3	*csu164b**
		375	203.3	*umc2060*
	211	39	216.29	*umc2388*
		93	216.01	*umc2113a*
		134	216.3	*bnlg1046**
		294	216.32	*umc1852*
		300	217.85	*umc27a*
		352	217.8	*umc1597**
	212	63	230.35	*umc1468*
		492	230.39	*bnlg557*
		500	230.39	*PCO135705*
		571	230.4	*umc2159*
		583	230.4	*mmc0351**
	213		231.5	unpublished
	214		232	unpublished
	215	23	235.6	*AY111142**
		201	235.62	*umc83b*
		397	235.64	*cpn1*
	216	86	242.6	*umc1048**
		16	242.59	*px13*
		285	242.62	*bnlg1700*
		342	247.6	*umc1447**
		348	245.5	*umc2294**
		579	247.62	*umc1163*
		749	254	*lim175**
		1231	257.8	*umc2295**
	218	874	260.2	*umc1315**
		1257	260.24	*umc1151*
	219	696	267.5	*umc2296**
		1008	267.7	*umc1935**
		1300	267.73	*umc1475*
		1550	271.5	*umc1692**
		1612	271.5	*umc1850*
	220	634	275.9	*gpm5*
		642	279.1	*umc2297**
		646	279.1	*umc1212*

Chromosome 5				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		696	275.9	*umc1609**
		768	279.11	*umc2073*
		781	279.11	*umc1784*
		840	279.12	*AY107414*
		899	281.2	*umc1355**
		964	281.21	*umc1274*
	221	173	285.49	*cat1*
		262	285.5	*umc1870**
	222	160	286	*umc1226*
	223	79	286.5	*umc1389**
		79	286.6	*umc1429**
		88	286.6	*umc1731*
		89	286.6	*umc1373*
		257	286.62	*a2*
		394	286.64	*umc2140*
		981	286.71	*gtc102*
	224	329	294.94	*cdo456c*
		762	294.99	*umc2400*
		838	295	*mmp58**
	225	539	297.5	*bnlg1902**
5.04		**606**	**301.6**	***bnl4.36****
		893	301.63	*umc2373*
		895	301.63	*umc1815*
		1410	307	*umc2298**
		1414	307	*umc1110*
		1418	307	*csu670*
		1487	307.01	*bnlg653*
		1532	307.01	*csu774(lhcb)*
		1980	310	*umc2299**
	226	160	309.82	*uaz275*
	227	180	309.82	*umc1629*
		221	314.1	*umc1591**
		646	314.14	*umc1224*
		912	315.2	*umc2300**
	228		314.4	unpublished
	229	171	315.72	*umc1563*
	230		315	unpublished
	231	92	316.26	*bnlg1287*
	232	66	316.53	*umc1860*
		476	316.54	*umc1283*
		934	313.3	*bnlg1892c**
	233	54	316.8	*umc2302**
		63	316.8	*umc2406*
		162	316.81	*umc1162*
	234	1247	317.6	*umc1060**
	235	102	320.1	*BE639933**
		678	318.79	*nfd108*
		1171	318.85	*dupssr10*
	236	154	336.39	*npi285d(cac)*
		733	336.47	*AY105205*
		1003	336.5	*AY110906**
	237	281	338	*AY105029**
		380	338.01	*umc1092*
	238	30	346.45	*umc1192*
		183	346.46	*PCO103687*
		600	346.5	*umc1348*
		600	346.5	*umc1349**
		1014	351.2	*AY109532**
		1053	371.2	*csu308**
		1053	368.4	*umc1221**
		1146	377	*csu600**

Chromosome 5

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		1151	377.9	*umc1966**
		1193	377.9	*incw1**
		1334	377.91	*umc2111*
5.05		1758	389.9	*mmc0081**
	239	46	394.4	*phi333597**
	240	174	392.7	*AY109682**
		190	397	*umc2026**
		349	397.02	*mmc0282*
		357	397.02	*umc1800*
		376	402.2	*mmp47**
		403	404.9	*umc1264**
		494	427.92	*serk2**
		644	427.93	*umc2386*
		778	427.94	*PCO060271*
	241	18	408.8	*PCO078116*
		18	408.8	*umc2303**
	242	84	410.8	*umc1155**
		138	410.8	*umc1687*
		189	410.81	*AY111089*
		209	410.81	*CL11475_1*
		217	410.81	*umc1502*
		323	413.8	*nbp35**
		324	413.8	*umc2086*
		324	413.6	*csu173**
		520	413.82	*PCO099796*
	243	25	415.78	*AY107329*
		27	415.78	*CL16923_1*
	244	368	435.96	*bnlg1847*
		407	435.96	*umc1853*
	245	27	467.14	*umc1722*
		260	467.17	*cdo400a*
		601	467.2	*AY110063**
5.06	**246**	**330**	**479.47**	***umc126a****
		331	469.39	*umc156b*
	247	31	470.1	*AY109938**
		258	470.12	*umc2072*
		346	476.6	*mmc0481**
		462	481.2	*umc54**
		566	479.7	*umc2305**
	248	25	488.4	*csu604b(trh)*
		31	488.4	*umc1752**
	249	309	493.5	*umc1524**
	250	52	493.5	*umc1680**
		87	492.7	*umc1941**
		121	493.7	*umc51a**
		463	493.73	*csu615a*
		536	500.7	*bnlg609**
		540	500.1	*umc2306**
		922	511.3	*rz567a(klc)**
		967	511.3	*PCO111982*
		1208	511.33	*umc2216*
		1240	516.3	*mmp169**
		1533	516.33	*umc49m*
	251	68	528.83	*umc2201*
		79	528.83	*gln4**
		252	528.84	*umc2198*
		407	528.86	*bnlg1346*
		439	538.49	*umc1537*
		575	528.88	*umc1646*
		591	528.88	*umc1375*
		681	528.89	*umc2013*
		687	528.89	*bnlg2305*
5.07	**252**	**27**	**536.6**	***umc108****
	253	331	590.39	*csu672a*
		369	590.4	*lhcb4*
		408	590.4	*bnlg1118**
		549	600	*umc1072**
		574	600.4	*AY110369**
		595	600.4	*bnl7.49d*
5.08		721	609.4	*bnlg118**
		884	625.8	*umc1792**
	254	15	643.6	*AY110182**
		158	643.61	*umc2143*
		217	656.7	*AY105910**
		255	656.7	*umc2136*
		359	664.3	*AW065811**
5.09		**441**	**669.4**	***php10017****
		497	669.41	*bnlg1695*
		497	669.41	*bnlg1711*
		574	669.41	*umc2209*
		697	676.7	*umc1153**

Chromosome 6				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
6.00	**256**		5	unpublished
	256	75	27.8	*bnlg1043*
		88	27.8	*umc2309**
		141	27.8	*umc2208*
		142	27.6	*umc2310**
			17.5	unpublished
	257	50	37.56	*umc2068*
		280	48.9	*umc1143**
		311	37.57	*csu782*
	258		30	unpublished
	259	29	60.87	*umc1883*
	260	159	63.58	*bnlg2243*
6.01		**368**	**66.4**	***umc85a****
		458	63.6	*AY110100**
		601	69.2	*umc1606**
	261	524	71.8	*mmp163**
	262	141	71.5	*umc2311**
		396	72.68	*umc1753*
		577	72.7	*bnlg1371**
		601	72.7	*bnlg426*
		602	72.7	*bnlg1165*
		904	75.8	*umc2312**
		1218	75.84	*bnlg1432*
		1218	75.84	*bnlg1600*
		1283	78.3	*bnlg1867**
		1299	78.3	*gpm8*
		1823	80.7	*umc1229**
	263	241	81	*umc1625*
	264	581	82.46	*umc2196*
	265	466	96	*uck1**
		666	98.4	*umc1444**
		951	98.4	*bnlg391*
		1308	98.4	*bnlg1433*
		1484	98.4	*AY107121*
	266	124	98.4	*bnlg1641**
	267	1731	98.43	*umc1498*
		1797	98.43	*csu243*
		1797	99	*umc2056**
	268	15	100.3	*uaz232b(sci)**
		112	100.31	*csu680e*
		270	99.3	*umc2314**
		548	110.4	*mmp10**
	269	60	98.6	*umc1133**
		726	98.4	*AY110213**
		1064		*rDNA5.8S*
		1209	112.38	*bnlg1538*
		1860	91.9	*umc2313**
		2013	98	*umc2074**
		2209	112.48	*bnlg1188*
		2326	314.6	*bnlg1174**
	270	501	116.16	*umc1195*
		648	116.17	*mez1*
		933	116.2	*mmp4**
		1226	120.5	*y1**
		1326	121.13	*cyc3*
		1388	121.14	*bnlg1139*
		1388	121.14	*csu146a(cdc48)*
		1664	121.16	*bnlg1422*
6.02	**271**	**225**	**124.94**	***umc59a***
		225	124.94	*umc1517*
		382	124.95	*saur1*

Chromosome 6				
BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		635	124.98	*umc1376*
		872	125	*umc1006**
		910	127.8	*umc1083**
		929	126.91	*mir2*
		930	127.8	*csu395a*
		982	133.4	*umc1656**
		1422	133.44	*bnlg2151*
		1439	145.7	*umc1257**
		1790	148.7	*bnlg2191**
	272	18	153.54	*umc1723*
		311	153.56	*sbp3**
	273	115	154.55	*umc2010*
6.03	**274**		166	unpublished
	275	329	166.6	*umc2316**
		361	167.6	*AY104775**
		409	166.8	*umc1887**
6.04	**276**	**142**	**181.9**	***umc65a****
		153	189.9	*umc1918**
		492	199	*umc1105**
		512	200.3	*umc1979**
		599	200.31	*si606044D05*
	277	42	203.2	*umc1857**
	278	34	204.1	*AY108825*
		57	204.1	*AY109804*
	279		205	unpublished
	280	311	211.3	*pl1**
		520	211.32	*agrr37a*
	281	299	228.89	*rz144b*
		383	228.9	*umc2006**
		1261	235.8	*umc2317**
		1330	235.81	*umc1614*
		1430	235.82	*bnlg1617*
		1659	235.84	*AY107053*
		1687	235.84	*tm20*
		1807	235.85	*bnlg1922*
		2084	235.88	*PCO152525*
		2089	235.88	*umc1795*
6.05		2393	244.9	*umc2319**
		2469	244.91	*AY105479*
		2474	244.7	*umc2318**
		2487	244.91	*AY107517*
		3234	253	*bnlg1154**
		3350	254.5	*umc1250**
		3458	254.51	*csu382a(cld)*
		3499	254.52	*PCO146525*
		3533	254.52	*umc1751*
		3551	254.52	*cesa2*
	282		269.8	unpublished
	283	18	277.09	*umc2055*
		140	277.1	*umc1413**
		487	277.13	*PCO134814*
		489	278	*bnlg2249**
		591	295.4	*umc2141**
		604	290.6	*AY110542**
		711	284.3	*umc1314**
		969	281.45	*csu360(elf1A)*
		1037	296.3	*AY110435**
		1060	296.3	*dhn1*
		1321	297.1	*umc1379**
		1475	297.12	*csu16b*
	284	286	302	*umc1388**

Chromosome 6

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
	285	102	310.68	*umc2065*
		223	310.69	*umc2040*
		229	312	*npi252**
		307	310.7	*AY110260**
		430	314.8	*AY109873**
		625	316.83	*mmc0241*
		715	317.77	*csu158b(eno)*
		718	317.8	*pdk1**
		719	317.81	*bcd454a*
		749	322.9	*umc2320**
		795	318.6	*AY110050**
		817	321.9	*AY110873**
		1029	318.91	*AY107881*
		1095	319	*umc2321**
		1212	319.16	*umc1462*
		1254	319.21	*umc1805*
	286		357.1	unpublished
	287	235	373.8	*bnlg1732**
		305	373.81	*si606039C09*
		462	373.82	*cdo312b*
		596	373.84	*bnlg345*
		646	373.84	*roa2*
6.06		**655**	**385.8**	***umc38a****
		729	388.7	*umc1912**
		751	391.4	*umc1859**
		936	394.1	*umc1762**
		968	393.9	*umc1463**
		1008	394.11	*CL10251_1*
		1060	398.5	*umc2162**
		1253	398.52	*umc2389*
		1309	410.3	*AY104923**
	288	100	420.4	*lim379**
		110	426.4	*AY105728**
		112	426.4	*umc1520*
		138	427.2	*AY105785**
		334	427.22	*umc2375*
		378	435.1	*umc2170**
		563	435.12	*hsp101*
6.07		**648**	**444.2**	***umc132a(chk)****
		677	444.2	*hdt103*
		799	450.7	*phi299852**
		845	452.68	*mlg3**
		845	450.7	*umc2123*
		877	466.5	*umc1490**
		1052	483.5	*umc2323**
		1177	501.2	*AY104289**
		1178	498.7	*AY109797**
	289	42	502.9	*umc1897*
		42	502.9	*umc2165**
		52	503.4	*bnlg1759a**
		83	427.53	*umc1350**
		111	504.8	*umc1248*
		155	510.6	*bnlg1740**
		156	510.6	*umc1779*
		156	513.8	*umc62**
	290	121	531.62	*umc1621*
		122	531.62	*bnlg1136**
		175	534.6	*mmp105**
		175	534.6	*umc1653**
		264	536.4	*agp2**
		430	536.42	*PCO068526*

Chromosome 6

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
6.08	**291**	198	548.7	*cdo202a(mcf)**
		223	542.7	*umc1127*
		238	542.7	*umc2059**
		319	544.5	*bnlg1521*
		319	544.5	*umc2324**

Chromosome 7

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
7.00	**292**	58	-6.3	*umc1788*
	293	54	2.7	*csu582**
		70	-0.89	*hsp3**
		241	13.8	*umc1241**
		254	13.94	*bnlg1367*
		356	27.4	*umc1378**
		356	27.2	*umc1642**
		383	27.4	*umc1378**
		444	45	*umc1694**
		471	47.8	*umc1426**
		553	53.3	*bnlg2132**
7.01	**294**	273	69.1	*AY104465**
	295	12	69.07	*umc1840*
	296	59	86.3	*AW308691**
		183	86.31	*hda110*
		325	86.33	*umc2364*
		363	92	*umc1159**
		392	93.41	*csu129*
		802	113.4	*mmp18**
		838	125.06	*o2**
		874	125.06	*umc2392*
		920	125.06	*dmt101*
		971	125.99	*his1a**
		1127	126.3	*umc1632**
		1359	126.31	*umc1428*
		1360	127.6	*umc2325**
7.02		1470	132	*asg34a(msd)**
	297	161	151.44	*bnlg1200*
		563	153	*umc1401**
		569	153.3	*umc1986**
		812	151.5	*AY109536**
		826	153.31	*cesa9*
		968	154.8	*umc2326**
	298	164	154.76	*sdg101*
		302	156.9	*umc1978**
		340	158	*umc2327**
		434	162.4	*AY105589**
	299	221	170.8	*crt2**
		913	170.87	*umc1339*
		995	212.31	*cdo407*
	300	940	176.8	*AY110576**
	301	124	178	*AY110473**
		573	178.03	*umc1666*
		573	181.3	*mmp187**
		610	181.3	*cncr2*
		898	181.33	*nbp1*
		1363	181.38	*umc1409*
		1397	181.39	*AY109061*
		1769	181.43	*umc1480*
		1935	181.45	*AY106170*
	302	408	180.5	*bnlg1094**
	303	205	183.4	*umc1879**
	304	587	186.3	*bnlg1247**
		872	186.33	*umc1433*
		966	188.1	*bnlg1380**
		997	188.1	*bnlg398*
	305	145	190.38	*vef101a*
		279	190.4	*bnlg1792**
	306	131	190.6	*bnlg2203**
		229	190.61	*pep4*
	307	494	192.5	*AY109809**

Chromosome 7

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		672	192.72	*hag102*
		759	195.6	*lim333**
		1079	195.63	*CL4745_2*
	308	48	204.8	*umc1932**
	309	1825	228.7	*AY109968**
		1992	228.72	*umc1036*
	310	155	244.3	*umc1983**
		155	246.3	*umc2142**
		198	249.1	*umc1929**
		465	252.4	*umc1787**
	311		252.6	unpublished
	312	188	252.9	*umc2092**
		502	252.94	*umc2057*
	313		255	unpublished
	314	217	258.4	*umc1393**
	315	120	261.49	*umc1585*
		257	261.5	*umc5b**
	316	242	273.8	*bnlg657*
		261	273.8	*bnlg1164*
		265	273.8	*bnlg1022a*
		374	273.81	*umc1881*
	317		297	unpublished
7.03	**318**	**20**	**298.37**	***asg49***
		87	298.37	*gst36*
		351	298.4	*umc1713**
		351	300.34	*php20569a**
		402	300.34	*umc1567*
		547	300.36	*bnlg1305*
		909	317.84	*umc1987**
		993	322.7	*bnlg1070**
		998	323.3	*bnlg434**
		1088	330.6	*npi394**
		1194	330.61	*ij1*
		1637	330.66	*umc1718*
	319	171	343.01	*bnlg339*
		233	343.01	*umc1275*
	320	339	347.19	*umc1333*
		414	347.2	*AY110374**
		461	347.2	*umc1481*
		656	351.4	*umc1660**
		983	365.4	*umc1408**
		1151	368.9	*umc1837**
		1187	368.9	*AY107911*
		1467	374	*rz404(ccp)**
		1559	374.01	*si614054G01*
		1616	374.01	*PCO101826*
		1661	374.02	*umc1841*
		1754	380.6	*umc1865**
		1798	381.5	*umc2328**
		1831	381.16	*umc1001*
		1837	381.2	*umc1134**
		2050	381.5	*AY109644**
	321	109	383.8	*bnlg2271**
	322	217	384.4	*umc2329**
		394	385.1	*umc1112**
		514	387.5	*umc1324**
		565	390.5	*umc1888**
		698	392.1	*bnlg1805**
		983	405.5	*umc1936**
		1049	405.5	*umc1301**
7.04	**323**	73	408.4	*umc2330**

Chromosome 7

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		73	408.4	*umc2331**
		172	410.5	*umc1710**
		321	412.1	*umc1251**
		344	412.1	*umc2062*
		376	412.11	*umc1684*
		395	412.11	*uaz199*
		616	429.2	*AY110023**
		683	430.5	*bnlg1666**
		1115	444.7	*umc1029**
		1163	444.7	*umc1342*
		1175	444.71	*umc1543*
		1271	444.71	*umc1944*
	324		454	unpublished
	325	30	471.38	*rip2*
		48	471.38	*csu5a*
		64	472.9	*phi328175**
		129	473	*AY110439**
		176	481.1	*umc1768**
		188	472.6	*umc2332**
		201	471.4	*umc1708**
		394	489.2	*bnlg2259**
		442	489.2	*AY108844*
		494	494.8	*umc1295**
		524	495.06	*umc1103*
		635	495.07	*rpot1*
		737	495.08	*csu904*
		926	518.9	*umc1412**
		931	517.5	*AW267377**
		988	530.63	*gpm2*
		1024	530.63	*PCO136133*
		1073	530.63	*PCO061754*
		1077	530.63	*umc1125*
		1230	530.64	*AY106318*
		1346	530.65	*chc101a*
7.05		1479	543.4	*mmp25**
		1487	547.7	*mmp17**
		1790	547.73	*umc2368*
		1815	547.73	*bnlg2328b*
		1863	541.11	*csu27*
		1874	547.74	*umc1671*
		1953	547.75	*umc2197*
		1997	547.75	*csu163a*
		2028	547.75	*umc1154*
		2040	547.76	*umc2222*
		2041	247.7	*umc1138**
		2046	547.76	*umc2379*
		2058	547.76	*cdo38b(ntp)*
		2095	593.4	*umc2333**
		2101	593.4	*umc35a*
		2101	593.4	*umc2190*
		2101	598.9	*umc1406**
		2101	600.2	*umc1407**
		2101	600.4	*umc2334**
7.06		**2222**	**608.2**	***umc168****
		2222	607.6	*umc1760**
		2288	611.5	*phi116**
		2335	611.51	*umc1799*
		2459	611.52	*umc1242*
		2509	618.4	*AY109703**
		2583	618.41	*oec6*

Chromosome 8

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
8.02	**327**	126	153.3	*umc1872*
		151	153.3	*umc1974**
	328	36	159.2	*cdo328**
		79	160.8	*umc1913**
	329	141	160.81	*umc1034*
		379	175.9	*csu329**
		550	175.92	*nfd110*
8.03		**553**	**175.92**	***asg24b(gts)***
		627	179.5	*umc1530**
		668	179.5	*bnlg1067*
		711	179.51	*bnlg669*
		727	179.51	*CL51477_1*
		917	179.53	*umc1778*
		933	191	*mmp120**
		1158	198.4	*umc2146**
		1158	197.1	*umc2147**
	330	310	199.1	*umc32b**
	331	334	200.3	*bnlg2082**
		337	200.3	*umc1868*
		950	200.35	*umc1236*
	332	675	202	*AW244963**
	333	111	203	*AY110450**
	334	271	203	*umc2353**
	335	71	204.8	*bnlg1834**
	336	226	204.82	*umc1360*
		293	206	*umc1157**
		402	206.6	*umc1904**
	337	53	231.2	*umc1470**
		84	232.9	*umc2355**
	338	404	240.7	*AY103821**
		508	240.71	*AW172071*
		792	240.73	*umc1487*
	339	156	269.66	*umc1471*
		202	269.66	*stp1*
	340	212	244.79	*csu742a(rpS7)*
		260	244.79	*umc1302*
		535	228.6	*umc1415**
		706	245.9	*AY105457**
		750	246.55	*umc2366*
		947	245.7	*bnlg1863**
		1279	254.8	*AY110032**
		1585	244.9	*umc2075**
		1699	267.74	*umc1615*
		1727	268.6	*AY109740**
		1816	268.6	*umc1427*
	341	111	273.8	*rps28*
	342	125	274.02	*umc1802*
	343	568	274.24	*AY110113*
		740	274.25	*umc1377*
	344	480	274.46	*umc1617*
		509	274.46	*oec23*
	345	39	274.89	*umc1473*
		703	274.9	*phi100175**
		809	279.9	*umc1735**
		912	282.7	*AY109626**
		931	284.6	*umc1457**
	346		285	unpublished
	347	53	289.8	*phi121**
		172	282.11	*tub2*
	348	27	295.25	*bcd454b*
		34	295.25	*mbd101a*

Chromosome 8

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		277	295.28	*bnlg1446*
		461	295.3	*rip1*
		474	295.3	*umc2154**
	349	257	304.2	*umc1460**
		464	307.59	*PCO147505*
		551	309.02	*csu9a(trf)*
		625	312.4	*umc1858**
		635	310.4	*AY110056**
8.04		1281	312.42	*AY107079*
	350	84	315.2	*umc1765*
		136	315.2	*bnlg2046**
	351	26	316.2	*pdc1*
		27	316.2	*pdc1*
		27	316.2	*cdo312a*
		158	316.22	*umc38b*
8.05	**352**	**212**	**329.4**	***bnl2.369****
	353	61	337.12	*rop7*
		74	337.13	*umc2367*
		229	337.14	*hox1**
		463	342	*AY104566**
	354	78	353.3	*umc1562**
		80	348.2	*mmp15**
		253	348.22	*umc1882*
		410	352.2	*umc1959**
		549	353.9	*umc1263**
		642	353.91	*mrp1*
		964	353.94	*umc1864*
		1087	357.9	*umc1846**
		1646	357.95	*umc2c*
		1738	367	*bnlg162**
		1738	367	*bnlg666**
		1738	366.8	*bnlg2181**
		1781	368.43	*bnlg1599*
		1784	368.53	*umc2378*
		1838	370.32	*csu829*
		1887	374.9	*umc1889**
		1907	372.6	*umc12a**
		1931	372.6	*umc1712*
		1931	372.6	*umc2401*
		1947	374.5	*bnlg1651**
		2215	377.7	*umc1340**
		2239	377.7	*hdt102*
		2804	377.76	*bnlg1812*
	355	27	381.68	*umc1670*
		30	382.9	*umc1777**
		176	381.7	*umc1316**
	356		382	unpublished
	357	244	215.6	*cdo1160a(kri)**
	358	127	388.71	*umc1121*
		748	388.77	*umc2175*
		794	388.78	*csu31a*
		1337	388.83	*umc2199*
		1528	388.85	*bnlg2289*
		1813	388.87	*umc1665*
		1945	388.89	*umc2210*
		2053	388.9	*umc17b*
	359		390	unpublished
	360	46	410.95	*bnlg1782*
		316	410.97	*umc1828*
8.06	**361**	57	412.88	*umc1141*
		173	412.89	*umc2031*

Chromosome 8				
BIN	**BAC FPC: Contig**	**CB**	**Genetic IBM**	**Locus**
		225	412.9	*umc1161*
		225	412.9	*umc2212*
		253	413.2	*umc1960**
		253	412.9	*umc2356**
		255	413.2	*sdg118*
		255	413.9	*umc1149**
		255	414.1	*bnlg1152**
		288	416	*mmp32**
		289	416	*bnlg240*
	362	81	432.37	*csu382b(cld)*
	362	143	432.37	*PCO079694*
		164	432.38	*sbe3*
		356	432.4	*umc2037*
		396	432.4	*umc1728**
		405	432.4	*umc2361*
		593	432.42	*umc2395*
		623	432.42	*mmc0181*
		646	439.6	*umc1905**
	363	145	455.1	*bnlg1031**
		170	456.17	*umc1724*
8.07		**222**	**459.2**	***npi268a****
		222	460.8	*bnlg1065**
		226	460.8	*CL9311_1*
		325	466.5	*umc1607**
		612	483.4	*bnlg1823**
		718	489.7	*psy2**
		718	486.9	*AY110569**
		848	494.7	*umc1268**
		963	494.71	*umc1384*
8.08		**978**	**509.8**	***npi414a****
	364	30	515	*mmp64**
		39	515	*umc1032*
		168	515.01	*umc2218*
		317	515.03	*umc2052*
		317	540.3	*umc1933**
	365	19	550.4	*AY110053**
		26	550.4	*bnlg1056*
	366	195	572.18	*gst1**
		195	580.1	*agrr21**
		245	596.4	*AY110127**
		327	609.1	*phi233376**
		333	608.1	*umc1663**
		364	621.6	*umc1638**
		654	632	*AY109853**

Chromosome 9

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
9.00	367		0.1	unpublished
	368	98	4.87	*AY112355*
		118	4.88	*umc1279*
9.01		534	5	*umc109**
		537	0	*umc1957**
		726	11.8	*bnlg1724**
		753	17.7	*umc1370**
		755	17.7	*umc2084*
		829	21.2	*umc1040**
		831	21.3	*bnlg2122**
		875	21.3	*umc2393*
		877	24.3	*umc1867**
		948	24.31	*bnlg1288*
		1118	41.68	*csu95a*
	369	17	62.3	*bnlg1583**
		17	62.3	*bnlg1810**
	370	134	64.7	*c1**
	371	105	74.8	*umc2335**
		201	80.3	*sh1**
		205	80.3	*umc2362*
		234	84.3	*umc1967**
		245	84.3	*stc1*
9.02		248	90.1	*bz1**
		309	90.11	*AY107496*
		370	90.11	*umc1958*
		373	95.8	*AY104252**
		375	95.8	*umc2078*
	372	43	101.1	*umc1764*
		72	101.1	*umc1170**
	373	68	101.1	*umc1430*
		131	101.11	*bnlg1372*
		286	101.12	*umc2130*
		286	101.12	*umc2219*
		383	125.7	*umc2336**
		562	131.1	*umc1636**
		816	131.12	*ss1*
		934	142.6	*bnlg244**
		1044	147.5	*bnlg1401**
		1359	164.15	*mgs3*
		1384	162.5	*mmp30**
		1386	162.5	*umc1037*
		1389	162.5	*umc1893*
		1390	162.5	*umc1033*
		1734	162.54	*AY105451*
		1757	162.54	*umc2213*
9.03		1962	192.17	*wx1**
		2016	193.2	*umc1634**
		2036	195.7	*umc1258**
		2082	196.4	*AY109570**
		2381	199.7	*umc1586**
		2468	199.91	*umc2128*
		2486	199.95	*gpm6*
		2508	202.3	*lim101**
		2509	202.3	*PCO061815*
		2676	200.4	*AY109816**
		2703	202.32	*d3*
		3009	219.4	*umc2338**
		3025	220.1	*umc2337**
	374	248	226.28	*csu321*
	375	447	226.3	*umc81**
		583	226.31	*bnlg127*

Chromosome 9

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
	377	75	230.95	*php20075b(ext)*
		260	230.96	*umc1420*
	378	413	241.55	*bnlg430*
		620	241.57	*umc2370*
	376	439	230.1	*umc1599**
		1109	230.16	*bnlg1626*
		1109	232.8	*umc1191**
		1339	240.5	*umc1271**
		1339	238.9	*umc2339**
		1345	238.4	*umc2340**
		1598	238.41	*AY107743*
		1598	238.41	*BE518809*
		2089	238.44	*gtd101*
		2206	238.44	*bnlg1688*
		2532	238.46	*si605086B11*
		2593	238.46	*gl15*
		2593	247.6	*umc1688**
		2593	244.1	*umc1691**
		2943	249.75	*cdo78*
		3272	252.3	*umc2087**
		3273	254.3	*umc114**
		3277	251.8	*umc1700**
		3349	254.6	*AY103770**
		3392	254	*umc1743**
		3610	253.7	*AW257883**
		3630	256.4	*csu147*
		3979	257.6	*umc1267**
		4497	257.66	*umc2394*
9.04	379	192	260.55	*bnlg1687*
	380	19	266.15	*sbp4**
		81	268.4	*lim166**
		343	268.43	*csu56d(ohp)*
		430	273.2	*bnlg1209**
	381	160	273.17	*umc1522*
	382	190	287	*bnlg1159b**
		342	298	*bnlg1012**
		788	298.04	*rz672b(cgs)*
		816	298.05	*umc1878*
		1015	308	*umc1492**
		1158	308.01	*AY110782*
	384	17	-86.18	*umc1120**
		129	311.9	*sus1**
		131	311.9	*AY109764**
		191	312.5	*mmp96**
	385	271	314.3	*mmp37**
		272	317	*umc38c**
		323	315.7	*umc2121**
		475	315.77	*csu694a(uce)*
		539	318.87	*bcd855f(ext)*
		861	318.89	*umc1771*
		944	318.89	*umc1519*
9.05		1092	322.6	*umc1078**
		1198	322.61	*umc1387*
		1239	322.62	*sod9*
		1582	322.65	*umc1654*
		1582	354.4	*mmp179**
	386	361	344.8	*umc1657**
		388	344.8	*umc1357*
		388	343.7	*mmp41**
		388	342	*umc1231**
	387	25	373.2	*AY110217**

Chromosome 9

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		436	373.24	*rgpr3235a*
		460	378.9	*umc2095**
		496	379.69	*umc1494*
		528	382.7	*csu634**
		560	381.1	*umc2341**
		604	384.8	*umc2344**
		607	384.9	*umc2342**
		686	385.2	*si687046G05*
		713	385.3	*umc2343**
		918	385.32	*csu651(rpL39)*
		1085	385.34	*csu59a*
		1091	385.34	*umc2371*
	388	25	386.22	*umc1417*
		26	386.22	*umc1417*
9.06	**389**	50	421.6	*gpm1*
		69	421.6	*mmp142**
		199	421.61	*umc1794*
		376	429.7	*AY109550**
		697	429.73	*AY107292*
		920	429.75	*umc76b*
		939	429.76	*bnlg1191*
		946	463.9	*csu93a**
		946	461.6	*umc2346**
		1006	491.99	*hb1**
		1271	492.02	*bnlg1525*
	390	35	526	*AY109819**
		52	526	*umc1310*
		103	526.01	*umc2207*
		130	526.01	*bnlg292a*
		223	534.2	*umc1789**
9.07		**245**	**535.95**	***asg12****
	391	20	536.8	*phi448880**
		162	538.5	*AY109543**
		179	538.5	*umc1804*
		290	551.3	*AY110382**
		325	554.4	*bnlg619**
		433	562.7	*umc2089**
		448	562.7	*umc2359*
		472	562.7	*bnlg1506*
		548	562.71	*bnlg1375*
		548	566.8	*umc2131**
		576	567.3	*umc1714**
		618	578.6	*umc2347**
		669	578.61	*bnlg128*
		785	587.9	*AY106323**
		1023	603.5	*umc1137**
		1031	603.5	*umc1942*
		1063	603.5	*umc1104*
		1102	603.51	*PCO127444*
		1257	603.52	*dmt103a*
		1281	695.45	*csu54b*
		1287	603.52	*rld1*
		1317	603.53	*umc1277*
		1320	637.1	*AI901738**
		1362	624.52	*umc1505**
		1399	633.2	*umc1982**
		1670	633.6	*bnlg1129**

Chromosome 10

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
10.00	**392**	225	-34.42	*umc2399*
		282	16.6	*umc1380**
		311	16.6	*umc1293*
10.01		**347**	**34.8**	***php20075a(gast)****
		348	30.9	*phi041**
		437	53	*AW330564**
		551	53.01	*rp1*
		604	53.02	*umc1291*
		692	53.03	*umc1319*
		705	53.03	*csu577*
		705	53.03	*cdo127b(pyk)*
		706	64.1	*AW225120**
		712	76.2	*umc2053**
		716	81.1	*umc2018**
		761	81.1	*bnlg1451*
10.02		**783**	**91**	***npi285a(cac)****
		787	91.4	*umc1152**
		874	104	*AY110360**
		910	106.98	*umc1432*
		937	97.9	*gdcp1**
		1044	120.1	*mmc0501*
		1046	120.1	*umc2034**
	393	242	143.3	*umc1337**
		242	148.9	*umc2114**
		269	136.66	*csu103a(aba)*
		281	148.9	*umc1582*
		281	143.5	*phi059**
		385	134.8	*AI795367**
		751	148.92	*PCO062847*
	394	452	160	*umc1863*
10.03		**482**	**160**	***umc130****
		691	160.02	*AY105872*
	395	236	168.2	*gcsh1**
		612	168.23	*umc1312*
		872	173.5	*lim2**
	396	127	179.66	*umc1866*
		339	179.68	*glu1*
	397	170	180.7	*umc1785*
		176	180.7	*umc1962**
		455	183.8	*umc1367**
	398	81	183.4	*bnlg210**
		1013	184.88	*chr109a*
	399	385	186.95	*bnlg1716*
		1226	186.99	*sdg108b*
		1351	187	*umc1381**
		1410	187.01	*gpm15*
		1699	187.03	*du1*
	400	286	194.5	*umc2067**
		289	194.84	*umc2017*
		294	195.4	*umc2016**
		608	195.43	*umc1938*
		1223	200.5	*AY110248**
		1279	200.51	*npi602*
	401		212	unpublished
	402	245	213.3	*umc1239**
		245	213.1	*bnlg1079**
	403		215	unpublished
	404	277	220.1	*AY112073**
		305	217.8	*bnlg1712**
	405	211	225.7	*bnlg640*
		211	228	*bnlg1655**

Chromosome 10

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
	406	255	228.3	*umc1336**
		375	228	*umc1739**
		419	228.31	*nac1*
	407	177	227.9	*umc2349**
		178	228.3	*umc2180**
		181	227.9	*csu276*
	408		228.5	unpublished
10.04	**409**	256	245.9	*umc1995**
		293	244.6	*umc2348**
		672	245.95	*umc1589*
		1030	248.2	*umc1246**
		1347	248.2	*umc1246**
	410	82	254.5	*AY110514**
		162	254.5	*cdo456a*
	411	665	253.35	*bnlg1526*
		761	253.4	*rz69**
		1786	253.89	*umc1827*
		1823	256.8	*AY109920**
		2535	260.12	*mgs1**
		2758	260.14	*PCO086427*
	412	52	271.3	*AY110365**
		142	271.31	*incw3**
		224	270.5	*mmp121**
		224	268.1	*mzetc34**
		539	280.7	*AY109698**
		929	273.6	*umc1911**
		1168	274.4	*umc1453**
		1478	283.5	*umc2350**
	413	112	290.9	*umc1697**
		308	287.9	*umc1330**
		605	299.4	*umc1272**
		605	299.8	*umc1648**
		613	299.8	*csu86*
		924	299.81	*hag103b*
		1017	295.9	*umc1115**
		1167	301.6	*umc2003**
10.05		1215	319.5	*AY110634**
		1289	309	*umc259a**
		1310	322.77	*umc1280*
		1425	322.77	*umc1507*
		1898	332.1	*bnlg1074**
		1907	327.3	*mmp12**
		2001	334.37	*umc2156*
		2084	334.37	*PCO087182*
		2131	335.5	*bnlg1250**
	414	12	344.8	*umc1506**
	415	23	375.78	*por2*
		23	375.78	*tip5**
10.06		117	380.5	*bnlg1028**
		146	380.5	*r1*
		146	380.5	*sn1*
		258	376.9	*mmp71**
		289	375.8	*umc1045**
		289	376.3	*bnl10.13a**
		353	380.52	*umc57a*
		394	380.53	*umc44a*
		858	355	*umc2043*
		880	380.58	*umc2221*
	416	234	399.57	*csu615b*
	417	182	410.6	*umc1993**
		182	412.3	*bnlg2190**

Chromosome 10

BIN	BAC FPC: Contig	CB	Genetic IBM	Locus
		217	412.3	*dmt102a*
10.07		**446**	**437.6**	***bnl7.49a(hmd)****
		514	444.8	*umc1196**
		570	449.3	*bnlg1677**
	418	56	450.8	*AY110016**
		237	464.6	*mmp181**
		237	466.4	*bnlg1839**
	419	17	483.69	*umc1249*
		105	483.69	*umc2203*
		162	483.7	*umc1640*
		169	483.7	*umc1877*
		169	483.7	*umc2172*
		192	483.7	*bnlg1450**
		212	483.7	*gln1*
		468	505.5	*umc2021**
		473	505.5	*crr2*
		473	505.5	*umc1645*
		569	505.51	*bnlg1518*
		575	505.51	*umc1113*
		598	505.52	*bnlg1185*
	420	194	525.03	*umc1038*
		202	527.3	*umc1556*
	421	261	509.9	*AY109829**

VIII. COMMUNITY SERVICES AND MATERIALS

This list is not necessarily complete. In many cases, a nominal cost-recovery fee is charged. Check the WWW sites listed to be sure of current status and procedures. Refer to MaizeGDB (http://www.maizegdb.org/cooperators.php) and the Stock Center (http://w3.ag.uiuc.edu/maize-coop/Maize-Genome-Projects.html) for updated links to maize projects which may provide community services or materials not listed here.

I. Genetic Mapping

I. A. Map coordinates for MAGI (Maize Assembled Genomic Island)
URL: http://magi.plantgenomics.iastate.edu/

I. B. IBM panel of stocks: materials and mapping computation service

- **Seeds for IBM -94 and IBM-302 panels.**
Maize Genetics Cooperation Stock Center
URL: http://www.maizegdb.org/cgi-bin/stockcatalog.cgi?id=1

- **DNA for the IBM-94 panel of stocks**
Maize Mapping Project (Missouri). Nominal fee.
URL: http://www.maizemap.org/dna_kits.htm

- **Map positions, IBM-94 panel**
CIMDE Community IBM Map Data Entry
Mapped by MapMaker software onto a framework with documentation, that includes public map scores)
With permission of authors, data are submitted to MaizeGDB
URL: www.maizemap.org/cimde.html

I. C. Radiation Hybrid Lines
Oat-maize lines.
URL: http://agronomy.coafes.umn.edu/cornpep/nsf/

II. Microarrays

II. A. Maize Oligonucleotide Array project.
57,452 70-mer oligo-nucleotides on 2 slides.
Also offers a hybridization service (June 2006 updated).
URL: http://www.maizearray.org/

II. B. Iowa State Plant Genomics
49, 280 oligo-nucleotides on 3 slides
http://www.plantgenomics.iastate.edu/maizechip/

III. Reverse genetics

III. A. TILLING
Point mutations from your sequence.
The Maize TILLING Project.
Till et al. Genome Research (2003) 13: 524-530
URL: http://genome.purdue.edu/maizetilling/

III. B. MU insertions from your sequence
MTM Maize Targeted Mutagenesis Database
URL: http://mtm.cshl.edu/

IV. Clones

IV. A. Public cDNA and BAC clones, libraries, filters
Arizona Genomics Institute.
This resource provides most of the clones, BAC libraries and BAC filters that are in the public domain and at a nominal cost.
URL: http://www.genome.arizona.edu/orders/

IX. GRAMENE: A GENOMICS AND GENETICS RESOURCE FOR MAIZE

P. Jaiswal[1], J. Ni[1], I. Yap[1], D. Ware[2,4], W. Spooner[2], K. Youens-Clark[2], L. Ren[2], X. Wei[2], C. Liang[2], S. Pasternak[2], W. Zhao[2], I. Tecle[1], B. Faga[2], P. Canaran[2], D. Ravenscroft[1], C. Hebbard[1], S. Avraham[2], T. Casstevens[3], E. S. Buckler[3,4], L. Stein[2], S. Mccouch[1]

[1]Department of Plant Breeding and Genetics, 240 Emerson Hall, Cornell University, Ithaca, NY, 14853, USA; [2]Cold Spring Harbor Laboratory, 1 Bungtown Rd, Cold Spring Harbor, NY, 11724, USA; [3]Institute for Genomic Diversity, Cornell University, Ithaca, NY, 14853, USA; [4]USDA-ARS NAA Plant, Soil & Nutrition Laboratory Research Unit, Cornell University, Ithaca, NY, 14853, USA

Maize is an important crop for the USA, with an estimated production of 300 million Mt harvested from about 30 MHa in 2004. This contributed about 40% of the total world production, but used only an estimated 20% of the world area harvested (http://faostat.fao.org/). In the US alone 2004 corn production was worth about 23 billion USD (http://www.ncga.com/). This enormous contribution of maize towards agroeconomics, both around the world and in the USA, demands a continuous improvement of the agronomic traits, such as yield, early maturation, disease resistance, tolerance to various abiotic stresses and improved nutritional and post harvest qualities. Currently the maize genome that codes for these agronomic traits is being sequenced and annotated (http://www.eng.iastate.edu/abstracts/viewabstract.asp?id=1821). The Gramene database (http://www.gramene.org) takes advantage of the known maize genetic information and genomic colinearity (synteny) with rice and other major cereal crops. Gramene provides researchers with an excellent platform for drawing comparisons between maize and other cereals (Fig. 1).

Figure 1. The Gramene web page available at http://www.gramene.org. Users can start with the 'Quick Search' by typing their query or by following the links provided in the 'Quick Start' section. For browsing the individual sections and datasets follow the links provided in the drop down menus that include search, genomes, download, resources, about and help. The 'Quick search" option is available on the top right side of the web page on all other web pages within Gramene website.

In addition to the comparative analysis tools, Gramene maintains curated datasets that include literature, maps (genetic, physical and sequence based), markers, genes, genomes, proteins, QTL, pathways and molecular diversity. Several of these will be discussed below. Although the Gramene database provides information on a range of grass species, the datasets, their presentation or accessibility via various modules described in this report are focused on maize only, and the description is based on release #21 (May 2006) of the database.

Gramene is a collaborative project between Cold Spring Harbor Laboratory and Cornell University. We actively work with maize researchers and the MaizeGDB (Lawrence et al., 2005) (http://maizegdb.org) to provide useful genetic and genomic information on maize. The information provided via the database is either shared from MaizeGDB or curated in-house using both manual and computational methods. It is freely available and web-accessible. The technological core of Gramene is the MySQL database management system, an open source relational database system that is stable and well supported. The database and curated datasets are available and can be installed for local use by following the instructions described in the installation document (http://www.gramene.org/documentation/gramene_installation.html).

Maps: Many geneticists and molecular breeders have an interest in exploring and comparing the genetic maps, genes and QTL from previously published literature. To enable researchers to query these existing datasets, the central comparative map search tool, CMap, can be accessed from Gramene's 'Maps' section (http://www.gramene.org/cmap/index.html). CMap presents a map as a linear array of interconnected features that correspond to either a single linkage group (in the case of a genetic map), a single contig (for a physical map), or a contig or scaffold (in the case of an annotated sequence). To set up a comparison between different map sets from either the same or different species and/or map types, the researcher first selects a reference map set, and then selects a reference map (chromosome, linkage group or contig) from within the set. This reference map serves as the basis for any comparison that one chooses to make (Fig. 2).

Currently the Maps module hosted a total of 17 maize maps (http://www.gramene.org/db/cmap/species_info?species_acc=maize) characterized into four types, namely physical (one), genetic (seven), Bin (one) and QTL (seven) maps. Except for the mapset 'Maize Bins QTL 2006' the other 6 QTL maps were curated by Gramene curators emphasizing the abiotic trait QTLs mapped on them. A quick

comparison of the Gramene Curated AGI FPC Oct 2004 physical map with various types of maps from several species can be viewed in the matrix format (http://www.gramene.org/db/cmap/matrix?map_type_acc=&species_acc=&map_set_acc=&map_name=&use_colors=1&hide_empty_rows=1&show_matrix=1&link_map_set_acc=cmf1104a&prev_species_acc=&prev_map_set_acc=&prev_map_name=). In comparison to the maize 'MMP IBM2 neighbors 2004' map it has about 2380 correspondences. With cross species comparison to the rice sequenced genome the number of unique hits (based on shared mapped markers) is about 24,391.

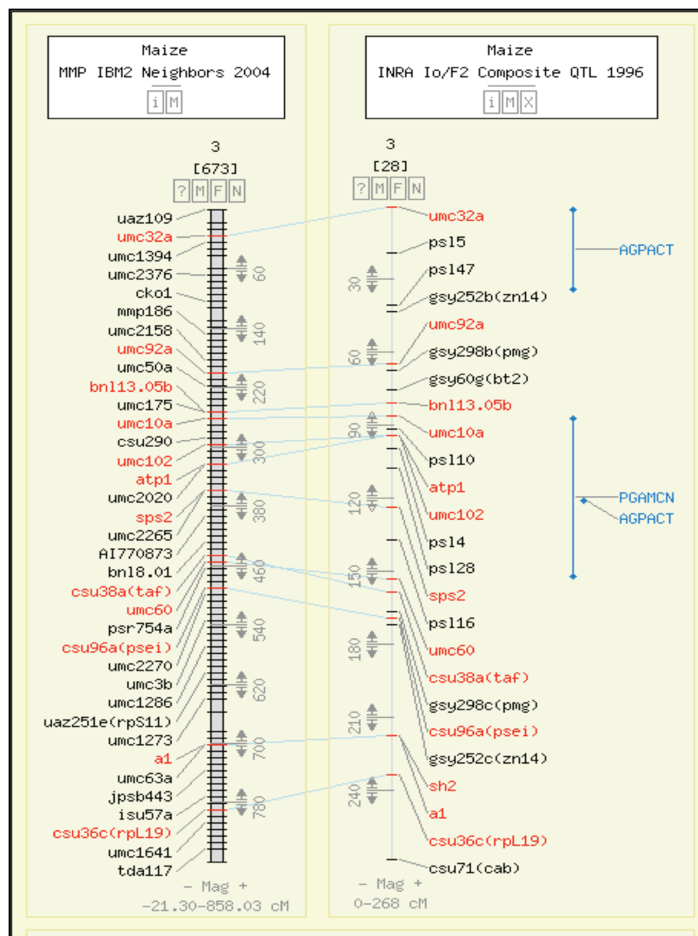

Figure 2. A comparative map display using the CMap tool. The maize maps compared are linkage group 3 from MMP IBM2 Neighbor 2004 and INRA Io/F2 Composite QTL 1996. The features (markers) in red suggest that a corresponding feature is present on both the maps. The labels and bars in blue present on INRA Io/F2 Composite QTL 1996 map are for the mapped QTL on two different traits phosphoglycerate mutase content (PGAMCN symbol) and ADP glucose pyrophosphorylase activity (AGPACT symbols). The QTL symbols displayed are acronyms assigned by Gramene curators for consistency in usage across several species. These symbols may be different from published symbols, which are recorded as synonyms.

Markers: Detailed information about markers mapped on the maps described above is provided by Gramene's 'Markers' section (http://www.gramene.org/markers/index.html). This module allows users to search the marker collection using one or more marker names, and a search may be refined by selecting the marker type (e.g. RFLP) and/or species (e.g. rice). A query for all RFLP marker types in maize gives 437 entries (http://www.gramene.org/db/markers/marker_view?marker_name=*&marker_type_id=3&species_id=4&action=marker_search). The marker details include marker name, synonym(s), type, species, the germplasm from which it was derived (if available), maps on which the marker can be found, and genome position(s) on the rice-japonica Nipponbare genome sequence, e.g. the maize marker CSU63 (Fig. 3) (http://www.gramene.org/db/markers/marker_view?marker_name=CSU63&marker_type_id=&species_id=4&action=marker_search).

Diversity: The genetic diversity database contains SSR and SNP allelic data, passport descriptions and associated phenotypes for maize germplasms. The major goal of this database is to be a resource for evolutionary, domestication, association, and genetic diversity studies on rice, maize and wheat. The maize diversity dataset presented on Gramene is imported from the Molecular and Functional Diversity of the Maize Genome project database (Zhao et al., 2006) available from http://www.panzea.org/. The introduction of the diversity database and links to various sections within Gramene will help users on potential applications such as germplasm management, marker assisted selection and DNA-based variety identification.

View Maize RFLP marker "CSU63"

Marker ID	4155
Marker Name	CSU63
Synonyms (14)	CSU063, CSU063a, CSU063b, CSU063c, CSU63a, csu63a(cdj), CSU63b, csu63b(cdj), CSU63c, csuh00063, M143, p-csu63 (GENBANK_ACCESSION), T12693 (GENBANK_ACCESSION), umc343
Type	RFLP
Species	Zea mays (Maize)
Germplasm	Unknown
Library	UNKNOWN
Description	

Mappings (13)

Species	Map Type	Map Set	Map	Name	Start	Stop	CMap Links	
Oryza sativa (Rice)	QTL	JRGP Nip/Kas F2 QTL 2000	3	M143	146.4	146.4	View on Map	Feature Details
Oryza sativa (Rice)	Genetic	JRGP RFLP 2000	3	M143	146.4		View on Map	Feature Details
Zea mays (Maize)	Genetic	IBM neighbors	1	csu63a(cdj)	862		View on Map	Feature Details
Zea mays (Maize)	Genetic	IBM neighbors	4	csu63b(cdj)	148.2		View on Map	Feature Details
Zea mays (Maize)	Genetic	IBM2 Neighbors 2004	1	csu63a(cdj)	973.97		View on Map	Feature Details
Zea mays (Maize)	Genetic	IBM2 Neighbors 2004	4	csu63b(cdj)	143.4		View on Map	Feature Details
Zea mays (Maize)	Genetic	UMC 1998	1	csu63a(cdj)	220.3		View on Map	Feature Details
Zea mays (Maize)	Genetic	UMC 1998	4	csu63b(cdj)	36.1		View on Map	Feature Details
Sorghum bicolor (sorghum)	Genetic	Paterson 2003	C	CSU063a	13.8		View on Map	Feature Details
Sorghum bicolor (sorghum)	Genetic	Paterson 2003	D	CSU063c	51.6		View on Map	Feature Details
Sorghum bicolor (sorghum)	Genetic	Paterson 2003	G	CSU063b	56.9		View on Map	Feature Details
Oryza sativa (Rice)	Sequence	Gramene Annotated Nipponbare Sequence 2006	Chr. 3	CSU63	32,649,212	32,649,293	View on Map	Feature Details
Oryza sativa (Rice)	Sequence	Gramene Annotated Nipponbare Sequence 2006	Chr. 3	CSU63	32,649,405	32,649,556	View on Map	Feature Details

Figure 3. The Marker detail view. The maize marker CSU63 was found to be mapped on several maps recoded in Gramene database. This includes a sequence based mapping on the rice genome map "Gramene Annotated Nipponbare Sequence 2006".

The database can be searched by germplasm accession number, accession name or marker/locus name. Searches can also be performed on the molecular diversity and phenotype, e.g. search the maize germplasm BOV 492 (http://www.gramene.org/db/diversity/diversity_view?search_for=BOV+492&object=&db_name=database_maize21&action=list) and view the details about it (e.g. http://www.gramene.org/db/diversity/diversity_view?action=view&object=div_passport&id=17). A user can find information on the experiment design as well as the alleles observed in it. For more detailed queries (not provided by web based search interface) you should use the standalone browser called The Genomic Diversity and Phenotype Connection (GDPC) (Casstevens and Buckler, 2004). Please visit http://www.gramene.org/diversity/gramene_gdpc.html to download and learn about the tool.

QTL: The 'QTL' section (http://www.gramene.org/qtl/index.html) facilitates the comparative study of QTL and their mapped regions in order to investigate colinear regions found to carry genes and QTLs identified in the maize and other grasses, and to investigate whether the same region/loci also contributes to similar traits and functions. Gramene does not currently curate raw QTL segregation data, but rather it emphasizes the presentation of basic QTL information such as the trait name, symbol, mapped position on the genetic, cited reference, and free-text comments, e.g. osmotic adjustment capacity QTL, AQFS427 (http://www.gramene.org/db/qtl/qtl_display?qtl_accession_id=AQFS427). The trait descriptions are mapped to a controlled vocabulary called the trait ontology (TO), which is a standardized vocabulary of traits to comparisons of phenotypes across species (Fig. 4). As of May 2006, the QTL module includes about 1700 maize QTL identified for 72 traits. Users can browse these traits and QTL by eight major trait families related to abiotic stress (113 qtl), biotic stress (6 qtl), fertility (none), anatomy (217 qtl), development (274 qtl), vigor (233 qtl), quality (143 qtl) and yield (495 qtl). The majority of the maize QTL were imported from MaizeGDB, but a handful on abiotic stress were curated by Gramene curators.

Genome: The maize genome (http://www.gramene.org/Zea_mays/) provides a graphical display of the annotations of the 504 full-length *Zea mays* clones deposited with GenBank as of 26-Jan-2006 (Fig. 5).

Annotations include various tracks displaying sequence alignments and details on the predicted genes, transcripts, peptides, ESTs, EST clusters (PlantGDB-TUGs, TIGR-GIs and MMP consensus), genetic markers (RFLP, SSR), flanking sequence tags (FSTs) from the mutant insertion lines, Hicot and Methyl filter reads and clusters from maize sequencing projects and other features of interest. This is a quick way to find the above datasets aligned to the gene(s) or BACs of interest.

In addition to the sequenced BAC views, the maize genome browser hosts an FPC physical map (Fig. 6) developed by the Arizona Genomics Institute (AGI; http://www.genome.arizona.edu/fpc/maize/). It is currently comprised of the 760 contigs from the AGI 25 Oct 2004 release.

The Ensembl synteny viewer (http://www.gramene.org/Oryza_sativa/syntenyview?otherspecies=Zea_mays) displaying the patterns of long-range synteny among the rice and maize genomes provides a useful comparative tool for users to find colinear regions of the rice and maize genomes as they search for genes, their functional orthologs and shared genetic markers. We constructed syntenic blocks between

Details for QTL "AQFS427" (osmotic adjustment capacity)

QTL Accession ID	AQFS427
Species	Maize (GR_tax:014450)
Published Symbol	qosmp1
Trait Symbol	OSADJCAP
Trait Name	osmotic adjustment capacity
Trait Ontology Accession:	TO:0000095
Trait Synonym(s)	OA osmotic adjustment osmotic potential
Trait Category	Abiotic stress
Linkage Group	1
Map Position	Maize-Maize Bins QTL 2005-1 (0.00-249.20 cM) [View On Map]
Genome Positions	None.
Comments	Located in centromere region This QTL was originally curated in MaizeGDB Database and the QTL map position was displayed on the maize bins map. Osmotic potential was measured to evaluate osmotic adjustment capacity and used for QTL analysis.
DBXRefs	Gramene Literature: Quarrie-SA Steed-A Lebreton-C Gulli-M Calestani-C Marmiroli-N, *QTL analysis of ABA production in wheat and maize and associated physiological traits*, Fiziol Rast, 41, 1994, pp. 565-571 MaizeGDB 102642

Figure 4. The QTL view. Details about the QTL on osmotic adjustment capacity (OSADJCAP) include, trait symbol, name, published symbol, linkage group, comments and citations. The hyperlinks from map position connect to the comparative map display.

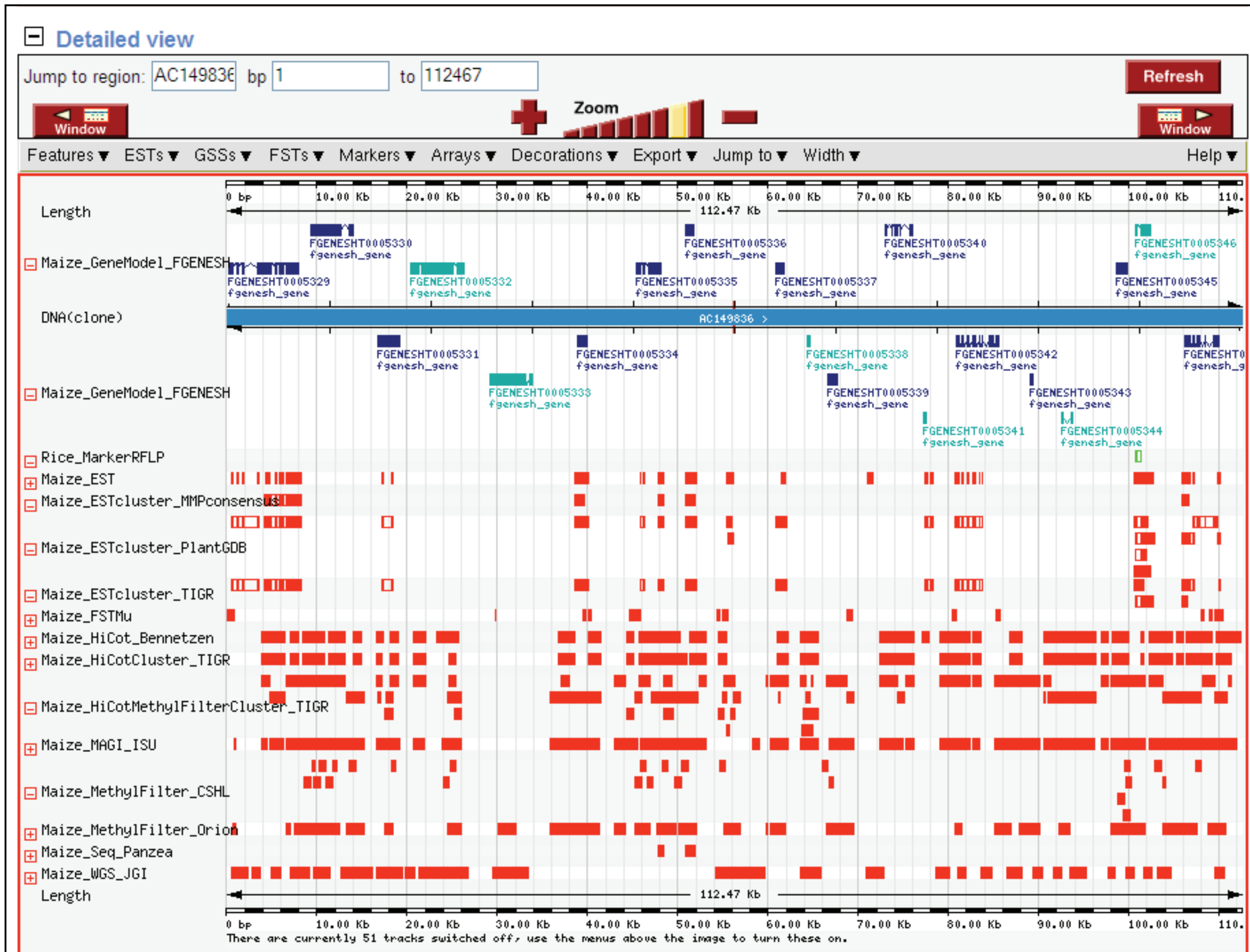

Figure 5. The maize genome browser view. Detail view of the sequenced BAC clone AC149836. The display allows zoom in and out, adding and removing tracks (follow drop down menus like features, ESTs, GSS, etc) and export the sequence and mapped features in various formats. The gene models in blue color suggest that there is a predicted ortholog from either or both rice and Arabidopsis.

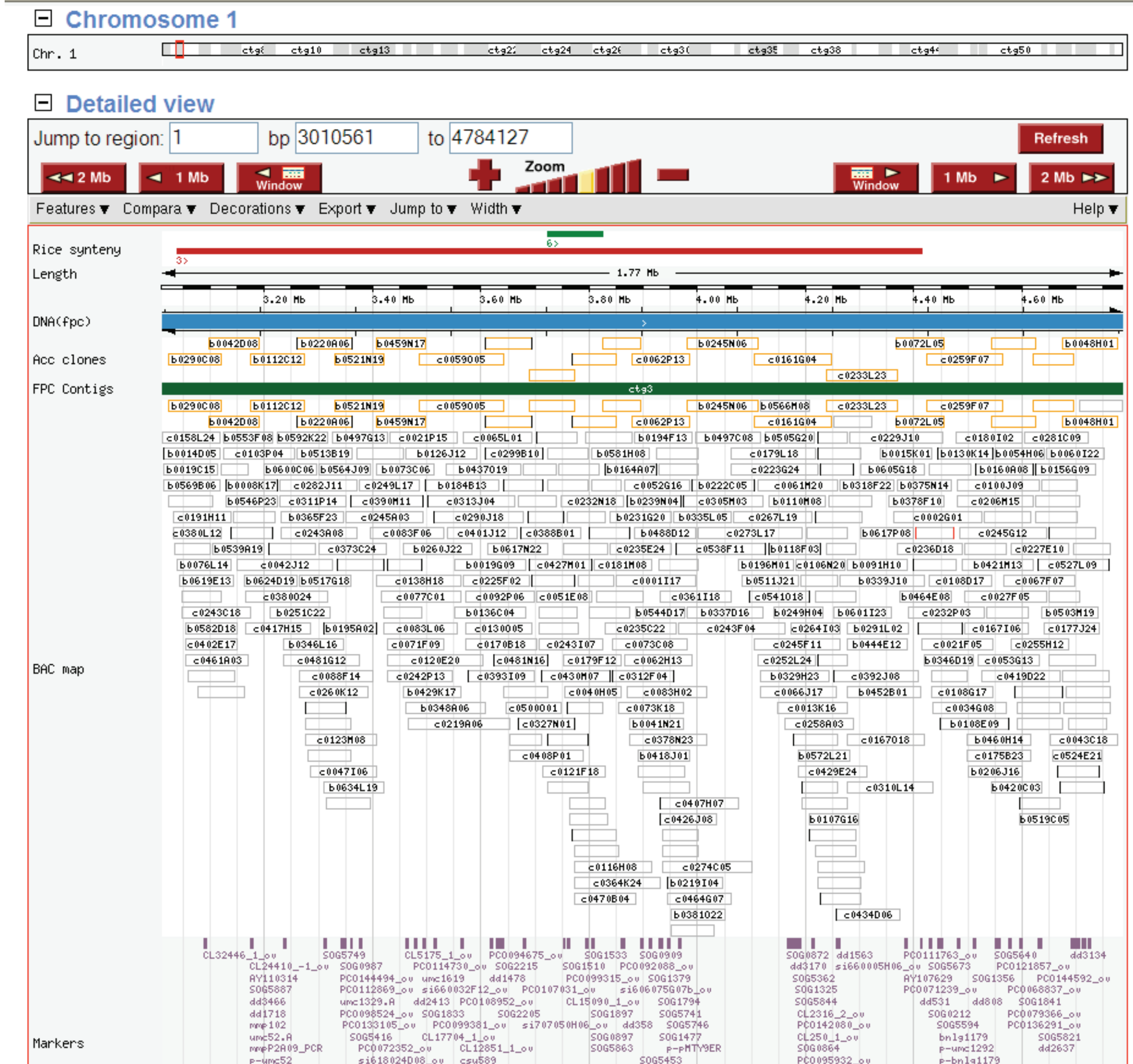

Figure 6. The contig view in the maize FPC map. The details include contigs, BACs, BAC ends, markers.

rice and maize by constructing a sorted pairwise list of locations of mapped overgo markers on the maize FPC map and then identifying their corresponding locations on the rice genome (Fig. 7) (D. H. Ware et al., unpublished data).

The annotated rice genome and its pre-computed comparisons with the maize and Arabidopsis gene models (genes) help users familiar with the function(s) or phenotype(s) of known gene(s) to traverse between these genomes and find the expressed, known and/or predicted gene sequence(s) based on either orthology or on gene function(s) (Fig. 8).

BLAST: The most frequently used tool on the Gramene website is the BLAST search (http://www.gramene.org/Multi/blastview). This allows users to perform similarity searches against sequence datasets that include bacterial artificial clones (BACs), BAC ends, proteins, ESTs, markers, genes (CDS), cDNAs, and FSTs such as Mu insertion lines from maize. Users can also query the maize sequence against the similar sequence datasets from other cereals, as well as genomes from rice and Arabidopsis, in order to find a gene, protein, region or phenotype of interest.

Genes: The 'Genes' section (http://www.gramene.org/rice_mutant/index.html) is a curated resource that in part provides publicly available information on genes from maize. It includes descriptions of genes, morphological, developmental and agronomically important phenotypes, and variants of physiological characteristics, biochemical functions and isozymes. Users can search for genes by their name, symbol or accession number. For example, a search for "tassel" yields as many as 16 genes with the word "tassel" appearing in either the

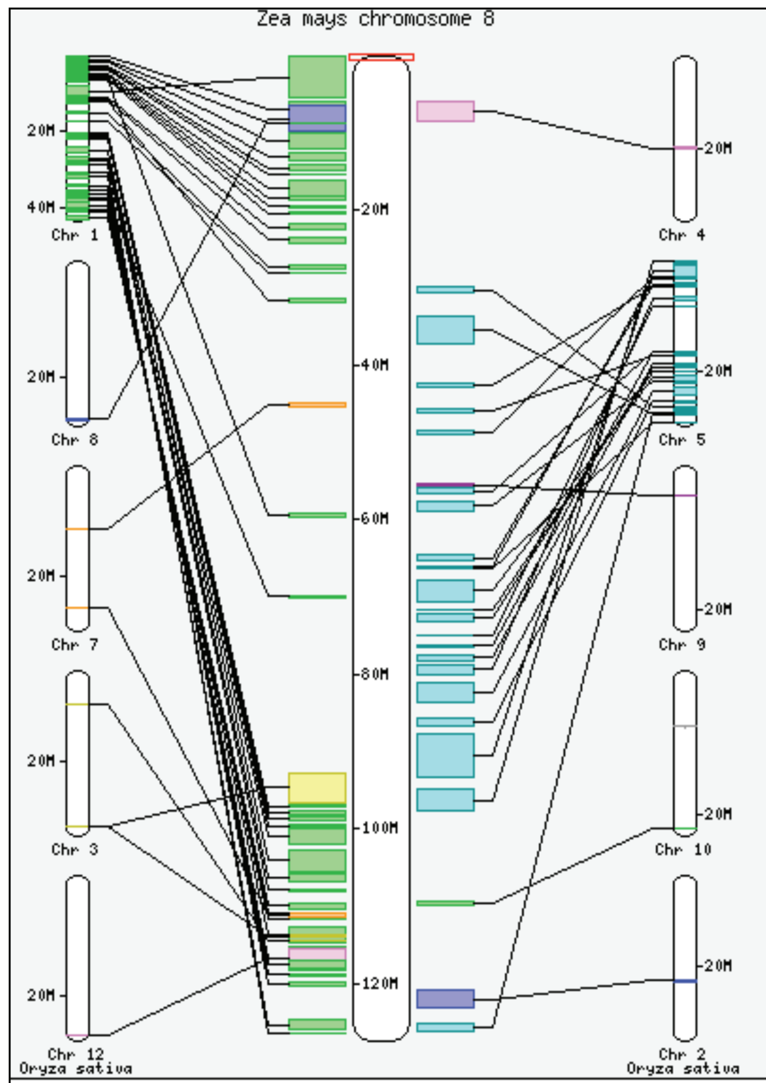

Figure 7. The macro level synteny overview between the Maize Chromosome 8 and rest of the rice genome.

gene name or the description (http://www.gramene.org/db/genes/search_gene?query=tassel*&search_field=name&gene_type_id=&species=2&query_submit=Search). As of May 2006, the database contained 6,676 maize genes, many fully annotated with phenotypic descriptions, map positions and citations. These were imported from MaizeGDB. In future we will collaborate with the MaizeGDB to provide associations to trait (TO) (Jaiswal et al., 2002) , plant structure (PO) (Jaiswal et al., 2005) and plant growth stages (GRO), similar to the information presented on rice e.g. slender rice (*slr*) gene (http://www.gramene.org/db/mutant/search_mutant?id=GR:0060842). This will enhance the comparison of phenotypes, expression and functional information among the orthologs from maize and other cereals e.g. maize D8 (http://www.gramene.org/db/genes/search_gene?acc=GR:0200107) as well as the height-regulating gene orthologs, wheat RHT and rice slender rice (slr) (Ikeda et al., 2001).

Proteins: This section (http://www.gramene.org/protein/index.html) provides curated information on approximately 4200 Swissprot-Trembl protein entries from genus *Zea,* of which the majority (4000) belong to *Zea mays* (maize). Protein entries are annotated using the Gene Ontology (GO) (Clark et al., 2005) for biochemical characterization. For example (Fig. 9), see the COX2 protein (http://www.gramene.org/db/protein/protein_search?acc=P00412). Information stored in this module is derived from Swissprot-Trembl protein sequence database, or generated by computational analysis that finds functional domains, transmembrane regions, signal peptides, etc. The report on functional characterization is supported with cited references along with a corresponding evidence code (experiment type http://www.gramene.org/plant_ontology/evidence_codes.html).

Ontologies: With the increasing demands of large scale genomic experiments that generate large datasets related to gene expression and phenotype analyses, the requirement for use of controlled vocabularies (ontologies) has become more apparent (Clark et al., 2005; Jaiswal et al., 2005). The ontologies are organized in categorical hierarchies of parent terms and child (more specialized) terms. For example the trait term 'plant height' has two parents, suggesting that it is a subtype of shoot anatomy and morphology trait and is also a sub type of height related trait (http://www.gramene.org/db/ontology/search_term?id=TO:0000207). This helps the user to find the

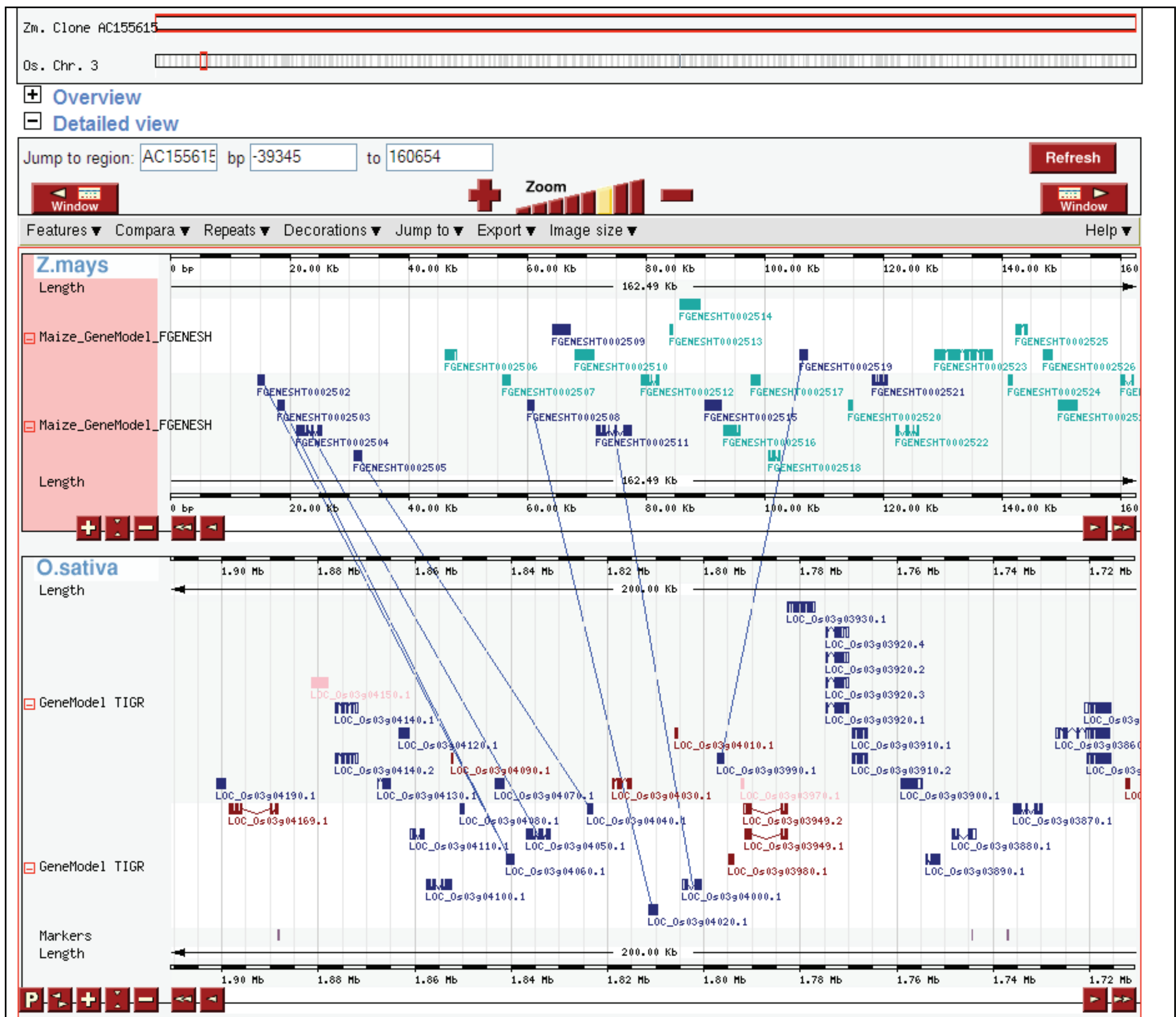

Figure 8. Micro level synteny view between rice and maize. These displays suggest the conservation of gene order within the two genomes.

associated genes and QTL either via the anatomy or the height-related trait path of the ontology tree and still get the same query result. For example there are 219 maize QTL associated to trait 'plant height'. To emphasize the use of such vocabularies to help users find genes, proteins, QTL, map sets and traits (Fig. 1, http://www.gramene.org/plant_ontology/index.html), we have adopted various ontologies including the gene (GO: Clark et al., 2005), plant (PO: (Jaiswal et al., 2005)), cereal plant growth stages (GRO), trait (TO: Jaiswal et al., 2002), environment (EO) and taxonomy (GR_tax) ontologies in our data annotation protocols.

User Assistance: To help users of our database, we provide pre-designed queries, glossaries and frequently asked questions (FAQs) sections. On-line tutorials (http://www.gramene.org/tutorials/) guide users through a step-by-step process to retrieve information from the database. General information about various cereal crop plants, including their genetic or evolutionary histories, production profiles, biology and commercial uses is also provided (http://www.gramene.org/species/index.html). For more information about Gramene, or to contribute suggestions, please contact Gramene at gramene@gramene.org.

We kindly request group(s)/person(s) who use the information derived from *Gramene* curation activities (EST alignments, mutants, comparative maps, gene and trait ontology annotations) to acknowledge the *Gramene* project contribution by citing the web address http://www.gramene.org/ and any of the appropriate Gramene publications (Jaiswal et al., 2002; Ware et al., 2002a; Ware et al., 2002b; Jaiswal et al., 2006).

This Gramene project was originally supported by the USDA Initiative for Future Agriculture and Food Systems (IFAFS) (grant no. 00-52100-9622) and USDA-Agricultural Research Service specific cooperative agreement (grant no. 58-1907-0-041). During 2004-2007 this

General Information about P00412			
Name	Cytochrome c oxidase polypeptide II		
Symbol	COX2		
Synonym(s)	Not available		
E.C. Number(s)	1.9.3.1		
Gene Name(s)	*COX2, COXII, MOX1*		
Organelle	Mitochondrion		
Best hits to TIGR rice gene models	Click here to generate a BLASTP query		
Accession Numbers	GenBank	SWISSPROT	
	CAA24094, CAA37046	P00412	
Database references	MaizeGDB CAA24094, CAA37046		
Organism(s)	Species	Cultivar	
	Zea mays	Golden Bantam early (GRIN)	

Associations			
Term Type	Term	Evidence	Evidence Code
Molecular Function	cytochrome-c oxidase activity (GO:0004129)	InterPro IPR002429	IEA
	copper ion binding (GO:0005507)	InterPro IPR001505	IEA
		InterPro IPR002429	IEA
Biological Process	electron transport (GO:0006118)	InterPro IPR002429	IEA
Cellular Component	mitochondrion (GO:0005739)	gramene.literature 7915	ISS
	membrane (GO:0016020)	InterPro IPR002429	IEA
		gramene.literature 8210	IEA
Keywords	Copper, Electron transport, Inner membrane, Mitochondrion, Oxidoreductase, RNA editing, Respiratory chain, Transmembrane		

Similarity to Other Proteins

Viridiplantae Green plants
-Embryophytes (plants)
Magnoliophytes (flowering plants)
-Monocots | Grasses | Rice | Maize | Sorghum | Wheat | Barley | Rye | Oat | Sugarcane
-Dicots | Brassicaceae | Arabidopsis | Fabaceae (Legumes) | Solanaceae | Cucurbitaceae
Others : Fungi | Metazoa
3D protein structures : BLink from NCBI | Sequence Annotated by Structure (SAS)

Protein Features		
Pfam (Info)	PF00116 ; COX2	All Members of this Family
	PF02790 ; COX2_TM	All Members of this Family
Prosite (Info)	PS00078 ; COX2	Sequence info. not available All Members of this Family
	PS50857 ; COX2_CUA	Sequence info. not available All Members of this Family
	PS50999 ; COX2_TM	Sequence info. not available All Members of this Family
Physio-chemical features	P00412	
ProtoMap (Info)	COX2_MAIZE	
Feature Type	Residues (From - To)	Evidence
Signal peptide	1 - 24	gramene.literature 7047
Transmembrane	42 - 64	gramene.literature 8210
Transmembrane	85 - 107	gramene.literature 8210

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Molecular and cellular biology, 1991, vol.11, pp4278-4281

Figure 9. The protein detail page showing associations to ontology terms describing its function and role in a biological process, evidences, citations and links to search for homologs and orthologs based on sequence similarity.

work is also supported by the National Science Foundation (NSF) award #0321685 and USDA-ARS. We are thankful to numerous collaborators, researchers and contributors from the cereal research community for sharing their datasets and for help in curation.

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X. MAIZE GENETICS CONFERENCE

<http://www.maizegdb.org/maize_meeting/2006>

Steering Committee Meeting Minutes
48th Annual Maize Genetics Conference
Asilomar Conference Center
Friday March 9, 2006

Jay convened the meeting at 9 pm

Attendees: Jay Hollick (chair); Anne Sylvester (co-chair); Wes Bruce; Tom Brutnell; Karen Cone (ex-officio); Monika Frey; Erin Irish; Marty Sachs (ex-officio); Jorge Nieto-Sotelo; Mary Schaeffer (Polacco) (ex-officio); Richard Schneeberger; Trent Seigfried (ex-officio); Marja Timmermans

Jay suggested keeping meeting minutes and volunteered the job to the meeting co-chair. Hearing no objection, Anne agreed to take notes.

Jeff Bennetzen from MGEC presented concept of 2008 50th anniversary meeting to be held in Washington DC area. Discussion:

- Venue for receptions could be Botanical Garden, NAIM, or AMNH
- Jeff will talk with GSA, NCGA and other groups about venue
- Anne and Jeff will submit for venue proposals, which are needed soon
- Local organizer still needs to be identified

Jay presented a Charter as a way to formalize steering committee purpose. Discussion:

- Corrections: clarify the name so that conference is added
- Charter should be made public to community by posting on maizegdb
- Anne noted that it would be helpful to also develop an annual timeline of activities so the new chair has clear guidance on what is needed, when
- Timeline can be developed and added next year
- Motion to accept charter and post on maizegdb approved

New steering committee members were discussed and voted on.

- Peter Rogowski, Steve Moose and Mei Guo will replace Jay, Monica and Wes.
- New steering committee will be announced Sunday morning.

Discussion about 2007 meeting

- Dates are March 22-25, Pheasant Run
- Tom Brutnell will be co-chair for 2007 meeting (chair for 2008 meeting)
- Speakers – Susan Lindquist has agreed to speak on Friday
- Three other plenary speakers discussed and approved
- Anne will invite the plenary speakers
- Meeting will include an annual report from the MGEC

Further discussion about the 2008 meeting

Discussion of possible future venues:

- 2009 - return to Midwest for 51st meeting
- 2010 – Europe? Barcelona or southern France as possibilities

Meeting finances

- Budget is at bottom; Asilomar was expensive due to poster tent (essential) and aquarium (well worth it)
- Need to raise registration fees
- Should meeting planning stay centered at Missouri? Definite advantages for now, but will need to reconsider if meeting continues to grow
- Should the meeting be made a foundation - not for profit entity, which would make contributions tax deductible – Karen will look into
- Should the meeting become a GSA run meeting? Problem is it would become more expensive. Perhaps Vicki Chandler or Karen Cone could look into the option

Issue of vendors: should there be vendors at all?

- SC voted this year to not include vendors in the 2006 meeting
- However, one vendor still showed up and set up a poster that was borderline between selling and telling – yet we did not get any income from it
- Suggestion was made to charge a significant fee for vendors
- $5000 could be charged with a cut-off for number permitted – the money could be used for scholarship
- **Conclusion:** revisit the issue and revote by email if (when) it comes up prior to the 2007 meeting

Meeting registration fees

- Expecting approximately same number at 2007 (approx 550 registrants)
- Fees should be raised to $150 for registration, since this is the first year we are starting out with a near deficit. Also, the 2008 meeting will be expensive.
- Perhaps special events should be charged extra fees in the future
- Would it be worth also submitting a meeting proposal to USDA? NSF continues to be supportive, and should we expect more money from another federal agency? May not be a good strategy. Suggestion was made to discuss it with program directors.
- **Conclusion:** raise fees next year, but exact amount will be determined after budget finalized

Issue of fund-raising and international scholars fund

- Jorge and Torbert Rocheford were recognized and thanked for their successful efforts to distribute scholarship funds to Latin American students
- The concept of scholarships for developing countries was strongly supported, but replenishing the fund is now needed
- The t-shirt sales this year will go towards next year's international scholarship fund
- It was pointed out that the scholarships should be awarded based on need
- Do we want to adopt fundraising as a regular meeting activity?
- T-shirt fundraising was successful this year, but do we really want to formalize it as another steering committee activity? There was weak support for formalizing it now.
- Pass the hat could continue, but it also should not be formalized. Some are more comfortable with that kind of activity than others – should not therefore make it a necessary steering committee activity – the ad hoc approach seems to work best
- But we could add a donation check box to the registration form, as long as it is clear that the donation is not currently tax deductible
- **Conclusion:** the steering committee will not adopt formal fundraising as part of its annual activity.

Review and revisit of meeting guidelines

- As requested from outside the steering committee, an annual MGEC report will be provided at every meeting
- There will be no repeat performances i.e. the same speaker will not present two years in a row, although the same lab can have sequential presentations
- Large labs should not be penalized by a rule that there is only one talk / lab
- Senior grad students, post-docs should get priority
- Should there be a representative from NCGA on steering committee? No – but we should make room each year in the agenda for an NCGA report

Discussion of poster contest

- All agreed a poster contest was a great idea, thanks to Jay.
- Couldn't be implemented this year but will work on for next year
- Discussion of whether it will be announced and who will judge posters
- Steering committee would oversee judging by grouping by subject matter, then chair and co-chair would select winners along with committee
- Issue of money awarded would depend on budget balance next year
- **Conclusion:** will revisit in November 2006 when planning for abstract submissions for 2007

Discussion of Allerton meeting

Jay adjourned the meeting at midnight.

CHARTER FOR THE MAIZE GENETICS CONFERENCE STEERING COMMITTEE

This document serves to identify, clarify and guide the organization and activities of the Maize Genetics Conference Steering Committee. Changes and amendments are expected as the Committee moves forward and its service to the scientific community evolves.

Section I: Purpose of the Maize Genetics Conference Steering Committee

The Maize Genetics Conference Steering Committee (SC) shall be responsible for the organization, content, and execution of the Annual Maize Genetics Conference (MGC). The SC shall work with the Maize Genetics Executive Committee (EC) to facilitate communication of community-based issues through the MGC.

Section II: Maize Genetics Conference Steering Committee Organization

The SC shall be made up of 10 voting members, including 1 from the EC on three-year terms, and voluntary members as needed to support SC activities. Each year the SC shall recruit new Maize Co-operators to serve on the SC. Each year the SC shall elect one of the 1st year members as co-chair for the SC. The co-chair will assume chair responsibilities in the subsequent year.

Section III: Maize Genetics Conference Steering Committee Affiliation

The SC shall maintain legal affiliation with an organization providing implements necessary to carry out contractual, fiduciary, and underwriting arrangements pertaining to the MGC organization and execution.

Section IV: Committee Activities:

The activities assumed by the SC include, but are not limited to:

1: Convening an annual SC meeting coinciding with the MGC to facilitate SC functions.

- A: A meeting agenda shall be distributed to all committee members.
- B: Minutes of the meeting taken by the current co-chair shall be archived

2: Fundraising to finance the MGC

- A: Meeting grant application(s) shall be submitted to federal agencies
- B: Donations shall be solicited

3: Advising and approving MGC activities including, but not limited to:

- A: Inviting speakers
- B: Selecting and coordinating venues
- C: Organizing the MGC program
- D: Fundraising
- E: Awards

4: Maintaining contemporary guidelines to direct decisions regarding but not limited to:

- A: Inviting speakers
  - i: Scientific merit
  - ii: Gender and topic balance
  - iii: Career development
- B: Selecting venues
  - i: International participation
  - ii: Inclusiveness
- C: Organizing the MGC program
  - i: Talk sessions
  - ii: Poster sessions
  - iii: Workshops
  - iv: Community forums
  - v: EC reports
- D: Awards
  - i: Poster contests
  - ii: Service recognition

This original version adopted by the SC on March 10, 2006.

Summary of Community Forum
48th Annual Maize Genetics Conference
Friday March 9, 2006

TALK BY JANE SILVERTHORNE, NSF
After the genome….

Learning from other genomic and post-genomic efforts
- Comments on the importance of workshops in developing tools
- Mt Fuji analogy – there's more than one mountain to climb and planning should be ongoing
- Need to re-evaluate at each milestone and even before the milestone

History of *Arabidopsis* sequencing effort
- 1990 - seminal plan that included international cooperation and coordination / plan for the db stock center – decision was made, for example, that full length cDNAs were important
- 1994 - NSF meeting
- 1996 - sequencing project
- 1998 - NPGI accelerated project
- 2000 - sequence completed ahead of schedule

History of *Arabidopsis* post-genomic effort and planning
- 1995 - planning for post-sequencing effort started
- 1998 - tools discussion
- 2000 - Salk meeting lead to 2010 program
- 2010 - program that included important midway checkpoints (see recent report http://www.nsf.gov/pubs/2006/bio0601/bio0601.pdf) and was driven by biology as well as tool development

History of rice sequencing effort was somewhat different because there was already an international and industry effort in place
- 1997 – sequencing project was already underway
- 1999 – planning session for functional genomics started early and lead to IRFGC (International Rice Functional Genomics Consortium)
- 2002 - first announcement from combined industry, US and international effort
- 2004 – "finished" genome announcement
- IRFGC – developed similar plan to *Arabidopsis* 2010 program but focused on agronomic traits, not just driven by biology

Lessons –
- Planning must be organized by scientists and the plans should be science-driven
- IP materials and data release policies must be spelled out
- IP clarity is especially for crop plant
- Plan needs to be flexible to accommodate new technology and new events
- Coordination of db activities is particularly important, especially possibility of incorporating tools from other countries
- Partnerships are most efficient and very important

Talk by V. Sundaresan
After the genome: Lessons from *Arabidopsis* and Rice

History of developing -*omics* since yeast was sequenced 10 years ago

Resources and tools that have been important for *Arabidopsis*
- Proper gene annotations
- Reverse genetics tools
- Expression arrays of all types (transcriptome and proteome)
- Full-length cDNAs
- Proteomic tools
- Stock centers with full availability and user friendly
- Unrestricted access -no MTA

Note that all *Arabidopsis* tools lead to the Salk lines, one of most important tools developed but others include: affy chips, 2hybrid screening tools, RNAi tools, VIGS, etc

For rice, same needs and same tools

- Stock centers have been less satisfactory for rice community because US (Arkansas) focuses on germplasm primarily
- Japan and Philippines also have centers but transport and access is restricted.

Road map of needs in post-genomics in rice

- Need to strengthen genetic resources
- Need to be able to translate research for breeders
- Need hi thru put phenotyping tools
- Need activation tagging lines
- Need fully efficient transformation of elite lines and decisions about which line to focus on.
- Need comprehensive stock center in US
- More international integration is still needed
- Accessibility still a major issue

For maize community – comparative genomics tools with rice very important.

Panel Discussion: see list of posed questions by panel members

Open Forum

COMMENTS AND DISCUSSION FROM MAIZE COMMUNITY

When projects end what happens to resources that have been developed?

- Community needs to be able to access and submit information
- Includes the need to simplify bioinformatics access
- The information is all out there but it is currently not centralized and have to go to individual and unique websites, learn all new tools each time – must be integrated
- There are many home pages but no guide to how to navigate, i.e. no single one stop shop that consolidates info

MaizeGDB as portal

- There needs to be more than links to other home pages, needs to be integration
- It is up to the researchers to establish and if there is a need, researchers should contact Carolyn Lawrence

Central field space

- Needed for smaller institutions and for those researchers who can't handle big growouts
- Need continued community phenotypic screens

NCGA comments

- Want to help maintain focus on agronomic significant tissues because NCGA needs the basic research to improve and increase production and ensure productivity
- What are the next genomes that need to be sequenced?
- How are the decisions made for what is most efficient? NCGA looks to the community for the next genome to be sequenced
- NCGA also needs to understand the connections between the basic science and the applications so they can bring specific examples back to Congress.
- How do we finally tie back to economic issues?

For comparative genomics, look to the human genome as a model

- Now there are 5-6 mammalian genomes available and effort now is to fill in diversity
- Now is the time to think abut having more cereal genomes since sorghum, brachypodium, rice, maize are all on the horizon
- But to accomplish this informatics resources need to be integrated so that cross genome comparisons can be made for agronomic traits, QTL etc
- Bioinformatics has been a major investment for human genomics, involved major integration issues

Stock center needs to be enlarged and supported

- More mutant lines are becoming available and the concern is that the stock center will be overwhelmed
- The stock center needs more resources to propagate, maintain and distribute

More on db issues

- Need to remember that TAIR is up to 23 people, so they can archive and also work on new bioinformatics tools
- db should include training for how to use the resources
- Need to deal with the fact that resources don't get integrated so the question is how to capture all the effort
- What should the relationship be between maizegdb and gramene?
- Should gramene develop further or should there be another centralized resource?
- Perhaps competition among two to three db is valuable to push the work forward (as was the case for human genome)
- Remember only 4 people associated with maize gdb and 12 associated with gramene

Need to develop full range of profiling arrays

- Need to augment standard transcription profiling with epigenetic profiling tools
- Other profiling arrays needed especially promoter, tiling arrays etc

XI. COMMUNITY SURVEY RESULTS

Maize Genetics Executive Committee (MGEC) Survey Results (172 responses)
Posted at MaizeGDB May 2006.
<http://www.maizegdb.org/mgec.php>

As follow-up to the open community forum held at the Maize Genetics Conference Asillomar 2005, a questionnaire was developed by the Maize Genetics Executive Committee (MGEC) and posted to cooperators from MaizeGDB. Results are summarized below.

A score of 1 was assigned for "highest priority".

Question 1
Prioritizing General Community Needs

1. High quality maize genome sequence annotation. (avg: 5.72)
2. Improved maize reverse genetics resources that allow investigator to move from sequence to seed. (avg: 6.72)
3. An improved maize database that allows investigator to move seamlessly between multiple genomic datasets and expression analysis. (avg: 7.12)
4. Improved maize transformation that is inexpensive, fast, and possible in multiple backgrounds. (avg: 7.16)
5. Resources for rapid mapping of all maize mutants. (avg: 8.66)
6. Functional studies that focus on individual genes, gene families or networks. (avg: 9.06)
7. High density markers (MaizeHapMap): SNPs for all genes and a catalog of genes not in B73. (avg: 9.35)
8. Enhanced capacity at the Maize Stock Center including increased seed storage space. (avg: 9.45)
9. Improved tools for quantitative genetics. (avg: 10.1)
10. Gene replacement tools. (avg: 10.2)
11. Support of training workshops in maize genetics, genomics, and bioinformatics. (avg: 10.8)
12. Further development of a maize global transcript profiling service. (avg: 11.0)
13. Proteomic tools and data for maize. (avg: 11.6)
14. Continued development of cytogenetic methods including chromosome painting techniques. (avg: 12.9)
15. Funded support for community field space. (avg: 14.0)
16. Other; see individual responses (avg: 14.8)

Question 2
Future Sequencing Strategies
Which survey sequences would be most valuable?

1. Full-length cDNAs (avg: 2.55)
2. ESTs (avg: 3.99)
3. Methylation filtered genomic sequences (avg: 4.20)
4. High-Cot genomic sequences (avg: 4.49)
5. Random shotgun genomic sequences (avg: 4.55)
6. Other; see individual responses (avg: 6.25)

Which lines would be most valuable to sequence after B73?

1. Mo17 (avg: 2.25)
2. W22 (avg: 3.72)
3. Other *Zea* species; see individual responses (avg: 4.52)
4. Other inbred line; see individual responses (avg: 4.91)
5. Other grass; see individual responses (avg: 5.15)

When comparing overall sequencing strategies, 52.3% of those surveyed stated that they preferred near-complete sequencing of one line over survey sequencing of multiple lines.

Question 3
Database and Annotation Issues
What features do you want to see in an improved community database?

1. Tools to navigate from maize sequence to map position and possible mutants or QTL (avg: 4.16)
2. Tools to navigate from gene to reverse genetics tools such as insertion sites (avg: 4.68)
3. Tools to navigate from maize sequence to homologous and syntenous sequence from other grasses (avg: 4.77)

4. Tools to navigate from maize sequence to a complete profile of expression studies (avg: 5.19)
5. Tools to navigate from maize sequence to homologous sequences in other species (avg: 5.62)
6. Increased interoperability between MaizeGDB and Gramene (avg: 6.14)
7. More tutorials on using existing database resources, both at MaizeGDB and at individual project sites (avg: 7.11)
8. Availability of a sequence browser such as Ensembl at/through MaizeGDB that supports and maintains user-contributed annotations in addition to automatic annotations (avg: 7.15)
9. Other; see individual responses (avg: 8.89)

For a community annotation pipeline
47.0% of the respondents indicated that centralized annotation efforts by a single bioinformatics group was their preference
37.7% of the respondents indicated that decentralized annotation where individual groups contribute annotation to a curatorial site was their preference
15.1% of the respondents indicated an alternative solution was their preference (see individual responses)

Optional Individual Responses to Question 1 - General Community Needs
(27 responses total)
Maize specific small molecule database (metabolomics)
Better support for computational biology
Improved software tools for maize curators
Studies of mechanisms of resistance of maize to pathogens
454 Sequence Multiple Diverse Maize Inbreds
Funding opportunity for pilot studies
Maize Activation Tagging resources for dominant phenotypes
Support for non-wet lab genetic analysis
Increased support for Gramene to provide end-user analytical tools for analysis of all cereal genomic sequences
Further development of off the shelf maize global transcript profiling platform
Improved affy chip, incorporation of quality checked data into MaizeGDB & PLEXdb
Agronomist trained in genomics to apply what we have learned to the field
Establishment of a metabolomics center and service (NMR and GC-MS and NIRS)
Better channels for communication & collaboration
Career development workshops for young scientists
Make important papers web-available when poorly accessible (e.g. Wilkes 1979)
More support for long-term public corn breeding programs
Affymetrix whole genome array
Richer BIOLOGICAL CONTENT in the maize database
Quantitative genetics
Complete maize genome sequence
Genetics of reproductive signs. Nuclear-cytoplasmic interaction.
History of Maize cooperators, contact with maize cooperators
Understanding cellular localization of maize gene products
MaizeGDB: capture the empirical data; a strong professional curation staff for maize and cereal genome peer-reviewed literature to capture the empirically confirmed information.
Maize genome evolution
Reverse genetics in rice

Optional Individual Responses to Question 2(a) – which survey sequences would be most valuable?
(28 total responses)
Selected BAC clones of gene rich regions (6 responses)
MPSS developmental profiles (3)
454 sequencing (3)
Finish B73 to completion (2)
Selected gene amplicons for diversity resequencing & transposon flanking sequences (2)
Organelle genomes from many inbreds and relatives (shotgun) (2)
Ab10 in addition to chromosome 10 from B73 and Mo17
BAC/EST/GSS contigs from multiple inbreds anchored to a genetic map
ESTs from Normalized cDNA libraries
PCR amplicons corresponding to maize genes

Repetitive sequences
Affy-style arrays to discover SNPs across diverse germplasm or RIL pop. members
Gene enriched sequences (methyl & Cot are equivalent)
Set of BAC ends optimizing genome coverage

Optional Individual Responses to Question 2(b) - Suggested Inbred Lines For Sequencing
(47 responses)
A188, tissue culture and transformation ability (7)
Gaspe flint - short flowering time inbreds will enable comparative adaptive studies (3)
The diverse germplasm lines being studied by Buckler/Doebley group. (2)
F7: european counterpart to B73 (2)
Oh43; another commonly used inbred line and a third heterotic group (2)
A619, mutants behave very differently in this inbred (2)
Mp313E (aflatoxin resistant inbred),
Iodent line (an expired PVP),
H99 (phenotypic penetrance has been altered)
NC89 and K55 (4x intermated population of 500 lines is being developed),
W23 (distinct from W22 and used by many on the west coast),
Michoacán 21 (best inbred for tropical lines),
CML247 (CIMMYT line with high market value but low (a)biotic stress tolerance -- commonly used in crop improvement programs for developing countries), Parthenogenetic maize lines-haploinductors,
M20W (good suppresser of many mutations),
Mo20W (drought and stress tolerance),
CML103 (a tropical source that is relatively early in the Midwest and results in reliable seed set),
PH207, recently public elite inbred line that is important progenitor to many current commercial hybrids
F2 (using INRA reference stock) as a representative of flint material, which represents an high divergence with dent material and has been used for genomic studies in France
27 linkage founders from NAM
The 16 progenitors of the Iowa Stiff Stalk Synthetic (BSSS). This would be a step toward understanding how genes respond to selection in populations. BSSS is the most economically important. pop
An inbred equidistant (by genetic distance) from B73 and Mo17
Other inbreds mentioned are P 165, KYS, KY21, popcorn, B37, W64A, Tzi8, CML69, P39, 4 Co63

Optional Individual Responses to Question 2(b) - Suggested *Zea* Species For Sequencing
(68 total responses. most just suggested “teosinte”).
17 supported sequencing *Z. parviglumis*.
5 supported sequencing *Z. diploperennis*.
3 supported sequencing *Z. luxurians*.

Optional Individual Responses to Question 2(b) - Suggested Grass Species For Sequencing
(43 responses)
12 suggested sequencing *Sorghum bicolor* (Important economically and phylogenetically, small genome, relative with desirable traits, closest relative that is a crop, sufficiently different from maize for numerous traits).
9 suggested sequencing *Tripsacum*. *T. andersonii* and *T. dactyloides* were suggested. (Closest genera to *Zea* to compare genome evolution with Zea)
4 suggested wheat (major polyploid grass species; Pooideae for comparative purposes)
2 suggested barley (major true diploid crop species, access to genes not tractable in maize)
2 suggested Brachypodium; it's a promising model
Switch grass- perennial, may have important agronomic properties
sugar cane(because of its economic importance).
foxtail millet, small genome, outgroup for maize and sorghum, rapid cycling, potential model
Streptochaeta from base of grasses for comparisons with rice and maize
Eleusine indica; fills gap grass taxonomy, is diploid, and has small genome
Joinvillia sp.-- outgroup to the pre-grass tetraploidy.
Coix; a close relative of *Zea*

Optional Individual Responses to Question 3(a) What features do you want to see in an improved community database?
(23 responses)
Some 15% of respondents listed other, but lower ranked priorities. Notably, many focused on enhancing content of biological information, in particular phenotypic, both mutant and QTL. This aligns with the top navigation tool priority. Many indicated a need for a single unified genome browser, easier to use than gbrowse should be the navigation tool target. Others indicated a need for better access to machine-readable formats; deposit of all project data into the community database; more flexible BLAST; archival/historical information; interoperability with other databases (e.g. TIGR; GO; TAIR).

Optional Individual Responses to Question 3(b) Community annotation pipeline
(26 responses)
Virtually all of the 15% 'write-in' respondents indicated a preference for an initial annotation by a single group, with support provided for updates by others in the community, with or without curation.

XII. RECENT MAIZE PUBLICATIONS

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Where’s the corn?
Asilomar, CA March 2006
Photo courtesy of Ed Coe

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SEND YOUR ITEMS ANYTIME; NOW IS YOUR BEST TIME

MNL 51ff. on line	MaizeGDB - http://www.maizegdb.org
Author and Name Indexes (and see **MaizeGDB**)	
Nos. 3 through 43	Appendix to MNL 44, 1970 (copies available)
Nos. 44 through 50	MNL 50:157
Nos. 51 to date	Annual in each issue
Symbol Indexes (and see **MaizeGDB**)	
Nos. 12 through 35	Appendix to MNL 36, 1962 (copies available)
Nos. 36 through 53	MNL 53:153
Nos. 54 to date	Annual in each issue
Stock Catalogs	Each issue, updates only after No 78, and MaizeGDB
Rules of Nomenclature (1995)	MNL69:182 and **MaizeGDB** (1996 update)
Cytogenetic Working Maps	MNL 52:129-145; 59:159; 60:149 and **MaizeGDB**
Gene List	MNL69:191; 70:99 and **MaizeGDB**
Clone List	MNL 65:106; 65:145; 69:232 and **MaizeGDB**
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Mitochondrial Genetic Maps	MNL 70:133; 78:151 and **MaizeGDB**

Cooperators (that means you) need the Stock Center.
The Stock Center needs Cooperators (this means you) to:

(1) Send stocks of new factors you report in this Newsletter or in publications, and stocks of new combinations, to the collection.
(2) Inform the Stock Center on your experience with materials received from the collection.
(3) Acknowledge the source, and advice or help you received, when you publish.

MaizeGDB needs Cooperators (this means you) to:

(1) Annotate your publications and enter new genes at **MaizeGDB**. Inquire about access to the community curation interface (under tools at **MaizeGDB**) using the contact form on each page at **MaizeGDB**.
(2) Look up "your favorite gene or expression" in **MaizeGDB** and send refinements and updates via the public annotation "button" at http://www.maizegdb.org.
(3) Compile and provide mapping data in full, including the ordered array of map scores for molecular markers or counts by phenotypic classes; recombination percentage and standard error. Provide as a short note to the Newsletter (preferred) OR **MaizeGDB** directly.
(4) Provide probe or primer information per http://www.maizegdb.org/probe.php; fingerprint data and fragment sizes are significantly useful to colleagues.
(5) Provide BAC-probe relationships for BACs on public physical map (http://www.genome.arizona.edu/maize), especially if probes have been genetically mapped. This information will be shared with the maize sequencing project.

May you find a Unique corn in MM!